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THE EFFECTS OF A SERIES OF POISONS ON ADULT AND EMBRYONIC FUNDULI.

By TORALD SOLLMANN.

[*From the Marine Biological Laboratory at Woods Hole and the Pharmacological Laboratory of Western Reserve University.*]

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I. Introduction.

THE original object of this research consisted in the attempt to localize the site of action of a series of poisons, by observing the stage of embryonal development at which the specific action would make its appearance. It was thought that this could be done by determining the just fatal dose at different periods of development. The attempt proved unsuccessful, on account of the very slow action of the majority of poisons on the fundulus eggs, which made it quite impossible to confine the effects to any particular period (see the second paper).

The research was, therefore, changed to a determination of the fatal doses at different ages, in sea water and distilled water. The effects

on the embryonal heart were also studied, as the material is well adapted to these observations. Whilst the results did not lead to any very important generalizations, the data appeared of interest in view of the adaptability and extensive use of Fundulus material in biological research.

A summary of the principal conclusions follows each section.

II. Methods.

In experimenting on the adult funduli, three fish, about 5 cm. in length, were placed in a finger-bowl with 150 cc. of the solution. The bowl was covered with a glass plate, leaving a little space for an exchange of air. Observations were made at intervals during a day. When the drug was administered in distilled water, the fish were first washed several times to remove the adhering sea water.

Numerous control experiments showed that the fish of this size usually remained normal for several days under this confinement. Specimens of Fundulus majalis and of Stickleback, as also large individuals of Fundulus heteroclitus, die within a short time from asphyxia, and were therefore not used. Specimens which showed ulcers of the nose and especially of the abdomen succumbed to considerably smaller doses and were not counted.

Occasionally one of the control specimens would die under the normal conditions of the experiment. This introduces a little uncertainty into those observations in which a partial fatality occurred; but it is generally easy to decide whether death was due to the poison or to other causes, by control experiments, by the onset of the symptoms, and by the rapidity of death.

The question must be considered whether the fatal effect is due solely to the poison and represents the true fatal dose; or whether it is due to the combined influence of poison and confinement. The latter is rendered unlikely by the general agreement between the adult specimens and those which were just hatched and in which the need for oxygen, etc. must be quite different. In so far as the observations are comparative, this question is of no importance, for the confinement factors were constant in all the experiments.

The recently hatched embryos and the ova were observed in Syracuse watch-glasses, with from 8 to 10 cc. of solution. These were stacked. Control specimens lived a week or longer.

The following *stock-solutions* were used in making the dilutions:

Hydrocarbon narcotics. — Chloral hydrate, 10 per cent in distilled water.

Salts. — Barium chlorid, 1 per cent in distilled water; sodium fluorid, 1 per cent in distilled water; sodium oxalate, 1 per cent in distilled water; potassium cyanid, 0.1 per cent in sea water.

Neutral principles. — Digitalin germ. Merck, 0.01 per cent in sea water; picrotoxin, 0.1 per cent in sea water; saponin, crude, Merck, 0.1 per cent in sea water.

Alkaloids. — Atropin sulphate, 1 per cent in 0.9 per cent sodium chlorid, and 1 mg. hypodermic tablets; caffein sodium benzoate, 1 per cent in distilled water; morphin sulphate, 4 per cent in 0.9 per cent sodium chlorid; morphin hydrochlorid crystals; nicotin, 1 per cent in 0.9 per cent sodium chlorid; pilocarpin hydrochlorid, 3 mg. hypodermic tablets; quinin hydrochlorid, 1 per cent in distilled water and in sea water; strychnin sulphate, 0.1 per cent in distilled water and in sea water; veratrin sulphate, 0.1 per cent in distilled water and in sea water.

III. Effects of Poisons on Fundulus heteroclitus, together with some Factors which influence the Toxicity.

Symptoms. — With all the poisons except strychnin the symptoms were those of paralysis. In adults these progressed as follows:

General Paralytic Phenomena. — Torpor. Slight inco-ordination of swimming movements. Weakening, and tendency to float on side, then on back. Swimming quite "groggy," sometimes on back. At times unable to swim, then recovered ability. Gradual disappearance of reflexes. Stoppage of respiration. Gasps. Death. Rigor mortis. The color of the skin often becomes darker and more vivid toward death.

In *strychnin poisoning* the torpor is preceded by exaggerated reflexes. When the table is tapped, tetanic contractions, especially of the pectoral fins, can be obtained far into the course of poisoning.

In morphin poisoning the skin was covered by a white scurf.

The recently hatched embryos exhibit even less variety of symptoms, as follows: Absence of spontaneous movements and lessened response to touching with the needle. Tail alone moves. Float on side or back. Rhythmic movements of fins and gills persist for a considerable time after all voluntary muscles are paralyzed. No convulsive effects were observed with strychnin. (This agrees with the observations of Pickering [1] [p. 457] on embryonal chicks.)

The transparency of these young fish permits the observation of

[1] J. W. Pickering: Journal of physiology, 1893, xiv, pp. 383–466.

the *heart*. This was made slow and irregular by strychnin, veratrin, digitalis, and quinin. It was scarcely changed by chloral, picrotoxin, and morphin.

The *blood* (in the newly hatched) showed post-mortem laking after chloral and digitalis; it appeared also as if strong solutions of chloral (1 : 100) caused some laking during life.

Time of appearance of symptoms and of death. — With the just fatal to twice fatal doses of the majority of poisons, both in adults and in the newly hatched, the first distinguishable paralytic symptoms are observed in about one to five hours. The paralysis is advanced or almost complete in four to nine hours. Death occurs in nine to eighteen hours in adult fish, in sixteen to thirty hours in newly hatched. The adult fish always die within twenty-four hours, if at all. With the newly hatched embryos, the voluntary muscles (except fins and gills) are also paralyzed within this time, although death may be deferred for several days.

The following poisons constitute exceptions to the general description:

The onset of the paralysis is *exceptionally slow* with the *caffein* (about six hours; death in about sixteen hours); and with *morphin* (onset generally in sixteen hours, exceptionally in two hours; death generally in twenty hours).

The course of the intoxication is *very rapid* with:

Digitalin. — Onset in one and one-half to two hours; death in about six hours (extremes, less than one hour to thirty-six hours).

Nicotin. — Onset in two minutes with moderately large doses; death within twelve minutes with large doses, two to thirty-six hours with just fatal doses.

Cyanid of potassium. — Larger doses in adults, onset within one minute, death within five hours. On newly hatched, it conforms to the general description.

With all the poisons the course of the poisoning is *materially shortened by increasing the dose*, beginning paralysis being noticeable in about half an hour, complete paralysis in half an hour to three hours, death in one to six hours, after large doses; but a considerable increase of dose (about five times the just fatal) is required to shorten the poisoning materially.

This is illustrated by the following condensed tables, which show also the ordinary limits of variation:

TABLE I.

TIME RELATIONS IN ADULT FUNDULI.

Strength of solution.	Beginning paralysis.	Advanced paralysis.	Death.
ONE PART OF STRYCHNIN SULPHATE IN SEA WATER:			
200,000	2½ hrs. × 5½ hrs.[1]	1½ min. × 5½ hrs.	2½ × 22 hrs.
100,000	4 " × 9 "	5½ " × 9 "	5½ × 9 "
33,000	5 min. × 1¼ "	5 " × 1¼ "	1 × 5 "
10,000	3 "	3 " × 15 min.	1 × 4 "
5,000	6 min. × 8 min.	8 " × 10 "	½ × 3 "
1,000	4 " × 7 "	4 " × 10 "	14 × 23 min.
VERATRIN SULPHATE, ONE PART IN SEA WATER:			
50,000	3 × 7½ hrs.	3 × 9½ hrs.	3 × 9½ hrs.
10,000	¾ × 2 "	¾ × 2 "	1 × 4 "
POTASSIUM CYANID, ONE PART IN SEA WATER:			
167,000	< 50 min.	< 1¼ hrs.	1½ × 5 hrs.
100,000	< 40 "	¾ × 1¼ "	1¼ × 2½ "
10,000	1 min.	1 × 22 min.	½ × 2 "
PICROTOXIN, ONE PART IN SEA WATER:			
250,000	> 4 hrs.	> 4 hrs.	9 × 22 hrs.
100,000	5 × 9 "	5 × 9 "	5 × 9 "
20,000	1 × 3 "	1 × 3 "	1 × 4 "
10,000	3 min.	¾ × 1 "	1 × 6 "

[1] The phenomenon occurs between the times indicated by these figures.

TABLE I (*continued*).

Strength of solution.	Beginning paralysis.	Advanced paralysis.	Death.
SAPONIN, ONE PART IN SEA WATER:			
25,000	< 2¾ hrs.	< 2¾ hrs.	2¾ × 5 hrs.
20,000	< 1 "	1 × 1½ "	1½ × 5 "
10,000	< 1¾ "	< 1¾ "	< 2½ "
EXAMPLES FROM RECENTLY HATCHED EMBRYOS.			
CHLORAL HYDRATE, ONE PART IN SEA WATER:			
400	2 hrs.	....	8 × 22 hrs.
100	8 min.	....	8 min. × 3 "
STRYCHNIN SULPHATE, ONE PART IN SEA WATER:			
200,000	1¾ × 4½ hrs.	4½ × 24 hrs.	6 × 22 hrs.
100,000	2 × 7½ "	7½ × 11 "	....
10,000	< ½ "	½ × 1 "	5½ × 22 "
1,000	< ½ "	< ½ "	1 × 4 "
			24 hrs. plus
			11 hrs. × 3 d.
			5½ × 25 hrs.
			1 × 6 "
VERATRIN SULPHATE, ONE PART IN SEA WATER:			
100,000	6 × 20 hrs.	20 × 29 hrs.	20 × 29 hrs.
10,000	14 × 50 min.	½ × 3½ "	3½ × 20 "
			29 × 42 hrs.
			3½ × 22 "

The course of intoxication is the same whether the poison *is administered in sea water or distilled water*; but the fatal dose may be different in the two solutions, as will be shown below.

In recently hatched fish a longer time intervenes between the complete paralysis (excepting the fins) and death, than is the case in adults. With potassium cyanid the first symptoms also appear

later than with adults. With these exceptions, the *age* does not influence the action, although it may modify the fatal dose. (Ova are not included in this conclusion.) The *size* of the fish appears to act only by producing asphyxia in confinement. *Sex* has no effect on the fatal dose or on the time of the symptoms or of death.

Recovery. — This does not usually occur if the fish are left in the solution; in other words, sublethal doses produce practically no noticeable effect; if plainly marked paralytic symptoms occur, these are almost invariably followed by death. The only drugs with which recovery was observed are potassium cyanid, saponin, chloral, and occasionally and temporarily, nicotin.

If the animals are *transferred to unpoisoned water* at the moment when paralysis is complete, prompt recovery is the rule. It was observed with cyanid, nicotin, and strychnin; but if even a few minutes elapse between the complete paralysis and the transfer, there is no recovery. This was seen with chloral, cyanid, nicotin, strychnin and veratrin.

Unequal fatality. — With doses near the fatal limit, the different individuals in the same bowl are often unequally affected. The inequality refers particularly to the time of the development of the symptoms and of death. This holds true of picrotoxin, quinin, and strychnin. In some cases, however, it was absolute; *i.e.*, whilst some individuals died, others remained apparently normal. A fatality of one out of three could doubtless be referred in many instances to injury or disease; but a fatality of two, with the third fish quite normal, is scarcely to be explained in this manner. This was seen especially with chloral, sodium fluorid and oxalate, potassium cyanid, digitalin, caffein, nicotin, and veratrin. It must be remembered that this inequality occurs only with doses very near the fatal limit. It is probably less common than the similar inequality observed with mammals and frogs. The inequality was uncommon with saponin and morphin.

Fatal concentrations. — Column five, Table II, shows that the concentration necessary to kill all the fish, exceeds that which kills two-thirds of the fish by from 10 to 200 per cent, commonly (in half the cases) by 33 to 50 per cent; the mean figures of all experiments and all drugs is 33 per cent. It is therefore possible to calculate one dose from the other with considerable certainty.

A small difference means that all the individuals have very nearly the same susceptibility, or that the paralytic and fatal doses lie

TABLE II.[1]

FATAL CONCENTRATIONS.

I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.
Poison.	Age of fish.	Solution in:	Concentration, 1 part of poison in:				The toxicity for $\frac{2}{3}$ is to the toxicity for $\frac{1}{3}$ as 1 is to:	The toxicity in sea water is to the toxicity in distilled water as (S):(D).	The toxicity for adult fish is to the toxicity for the recently hatched as (A):(H).
			Maximum concentration which was never fatal.	Minimum concentration which was always fatal.	A fatality of 2 out of 3 lies between:	The av'ge concentration for $\frac{2}{3}$ fatality is:			
Chloral	A	D	800	200	500 and 250	375	1.88	1:1	1:1
Barium chlorid .	A and H	D	10,000	5,000	7,000 and 5,500	6,000	1.2	..	1:1
Sodium fluorid .	A and H	D	100,000	15,000	....	20,000	1.33	..	1:1
Sodium oxalate .	A and H	D	?(100,000 = $\frac{1}{4}$ 20,000 = $\frac{1}{10}$)	5,000	10,000 and 5,000?	7,500?	1.5	..	1:1
Potassium cyanid	A	S	500,000	167,000	....	250,000	1.5	1:2	$6\frac{1}{2}$:1
	A	D	?	375,000	....	500,000	1.33		
	H	S	250,000	25,000	67,000 and 25,000	40,000	1.5	1:2	
	H	D	(250,000 = $\frac{1}{3}$)	50,000	100,000 and 50,000	75,000	1.5		
Digitalin . . .	A	S	>350,000	100,000	250,000 and 200,000	200,000	2.0?	1:>$1\frac{1}{4}$	1:$1\frac{7}{8}$
	A	D	....	>250,000	....	>250,000	?		
	H	S	700,000 (450,000 = $\frac{1}{3}$)	300,000	400,000 and 350,000	375,000	1.25	....	
Picrotoxin . . .	A and H	S	(250,000 = $\frac{1}{3}$) 150,000	<100,000	....	100,000	?	1:2	1:1
	A	D	(250,000 = $\frac{1}{3}$)	175,000	....	200,000	1.14		
Saponin	A	S	50,000	25,000	....	38,000?	1.5	2:1	1:2
	A	D	>25,000	<20,000, probably 12,000	....	19,000			

Saponin	H	S	100,000	50,000	....	75,000?	1.5	2:1	1:2
	H	D	50,000	>15,000, probably 25,000	....	38,000			
Atropin	H	S		<1,000					
Caffein	A	S	100,000	1,000	50,000 and 10,000	45,000?	....	>1:1?	5:1?
	A	D	<25,000	<25,000	....	?	....		
	H	S	50,000	<10,000	30,000 and 10,000	<10,000	....	....	
	H	D	<75,000	<75,000	....	?	....	....	
Morphin . . .	A	S	500	<250, probably 190	....	250	1.33	1:2	1:1½
	A	D	>600	375	600 and 500	500			
	H	S	>1,000	333	>1000	450		1:>1	
	H	D	>375	<375	....	?			
Nicotin	A and H	S	<1,000,000	80,000	200,000 and 100,000	125,000	1.5	1:1?	See below.
	A	D	....	....	175,000 and 110,000	140,000?	....		
Pilocarpin . . .	H	S	>10,000	>10,000					
Quinin	A	S and D	(15,000 = ½)	6,000, probably 9,000	....	10,000	....	<1:1	See below.
	F	S and D	(6,000 = ½)	4,000	....	4,500	1.1		
Strychnin . . .	A and H	S	(400,000 = ½) (250,000 = ¼)	<200,000	(200,000 = ½)	225,000	1.1	<1:1 (1:1.5?)	1:1
	A	D	>300,000	>300,000	....	350,000?	....		
Veratrin . . .	A and H	S	(500,000 = ½)	100,000	250,000 and 125,000	175,000?	1.75?	1:3.5?	See below.
	A and H	D	....	>375,000	500,000	500,000	1.33		

[1] All details are omitted, as well as the individual experiments which were made, only the extremes, derived from a very considerable number of experiments, being given.

A, adult fish; H, recently hatched fish (from 1 to 14 days old); F, hatched one or two days; S, sea water; D, distilled water.

The concentrations signify one part of poison to the stated number of parts of water. (For instance, Chloral AD800, means that adult funduli were placed in a solution of 1 part of chloral in 800 of distilled water.)

The concentrations in Columns IV, V, VI, are those actually observed. When these are not sufficiently close to the probable limits, this is emphasized by the signs > (more than) and < (less than). In Column IV, accidental fatalities are enclosed in parentheses. For instance, under Digitalin there is seen (250,000 = ½), signifying that one fish out of three died in the 1:250,000 solution, but that the death was probably not due to the poison.

close together; this is seen with picrotoxin, quinin, and strychnin; whilst chloral, digitalin and veratrin show a large difference between the two doses.

Column six compares the *toxicity in distilled water and sea water*.

The toxicity is generally greater (*i. e.*, a lesser concentration is fatal) in distilled than in sea water. The only exceptions to this rule are chloral and probably nicotin, in which there is no difference; and saponin and perhaps caffein, which are more toxic in sea water (barium chlorid and sodium fluorid and oxalate could not be compared).

The ratio of the toxicity in sea water to the toxicity in distilled water is shown by the following:

More toxic in distilled water.	Same in distilled and sea water.	More toxic in sea water.
Potassium cyanid 1 : 2.	Chloral.	Saponin 2 : 1.
Digitalin 1 : > 1¼.	Nicotin (?).	Caffein (?) > 1 : 1.
Picrotoxin 1 . 2.		
Morphin 1 : 2.		
Quinin 1 : > 1.		
Strychnin 1 : > 1 (1 : 1.5?).		
Veratrin 1 : 3 5 (?).		

The poisons which are more toxic in distilled water differ widely in chemical and physical properties.

The relative toxicity in distilled water and sea water is evidently not dependent on the chemical nature of the poison, for the different classes are pretty uniformly distributed over the three groups.

It may be assumed that distilled water increases the toxicity either by hastening absorption, or by lessening some other factor of resistance. Speculation as to this would be idle in the absence of specific data. In the case of saponin, however, a very probable theory may be formulated: Saponin generally increases the permeability of membranes. Since the concentration of the fundulus blood doubtless lies much closer to distilled water than to sea water, it is easy to understand how an increase of permeability would be more harmful with sea water than with distilled water.

Column ten shows the influence of age on toxicity. The ratio of the toxicity for the adult fish (A) to the toxicity for the recently hatched fish (H) is:

Essentially the same for both.	More toxic to H A : H ::	More toxic to A A : H ::
Chloral.	Digitalin 1 : 1⅞.	Potassium cyanid 6½ : 1.
Barium chlorid.	Saponin 1 : 2.	Caffein 5 : 1 (?).
Sodium fluorid.	Morphin 1 : 1¼.	Nicotin) see next
Sodium oxalate.		Quinin) paragraph.
Picrotoxin.		
Veratrin (?) (see next paragraph).		

The toxicity remained constant for two weeks after hatching, with the following exceptions:

Toxicity for.	Freshly hatched.	Week old.	Two weeks old.	Adult.
Nicotin	1.	2.	..	1⅞.
Quinin	1.	2.	..	2.
Veratrin	1.	2½.	2½.	1.

These figures were drawn mainly from the toxicity in sea water; but the ratio is the same in distilled water, even in those cases in which the toxicity is different in the two media. For example:

TOXIC CONCENTRATION OF SAPONIN.

	For adult fish.	For recently hatched fish.
In sea water	1 : 38,000.	1 : 75,000.
In distilled water	1 : 19,000.	1 : 38,000.

I would not, at this time, risk any explanation for the variation of the toxicity with age. It is quite probable that the explanation varies for the different drugs.

The simultaneous application of several poisons. — The abundance of the Fundulus material makes it eminently suitable for studies on the antagonism and synergism of poisons. The essentially equal absorption (as evidenced by the almost uniform time required to produce death) is also a favorable factor.

Mr. G. E. Fahr made some of these experiments under my direction. The results are sufficient to indicate the possibilities of the subject; but the investigation would need to be carried much farther to yield practical results.

The experiments consisted in administering strychnin with the series of poisons, always dissolved in sea water. Embryos a week old were used, with the technic previously described. The results are given briefly in Table III.

TABLE III.

FATALITY OF COMBINED POISONS.

I.	II.	III.	IV.	V	VI.	VII.	VIII.	IX.	X.
DRUG.	Concentration just fatal to $\frac{2}{3}$ of the fish, 1:	Concentration used, 1:	FATALITY.						
			Drug alone.		Drug with strychnin; concentration of strychnin equals:				Net result of the addition of strychnin to the toxicity of the drug.
			Extremes of fatality.	Average fatality.	1:1,000,000	1:500,000	1:333,333	1:250,000	
Chloral . . .	375	1000	0[3]	0	..	$\frac{0}{4}$[1]	..	..	None.
		667	0 to +[2]	$\frac{1}{5}$	..	..	$\frac{0}{5}$	..	
		500	$\frac{1}{5}$ to +	$\frac{2}{5}$	..	..	..	$\frac{2}{5}$	
Pot. cyanid . .	40,000	400,000	0	0	$\frac{0}{3}$	..	..	..	Apparent increase, but the data are somewhat uncertain. The conclusion is probably correct.
		200,000	0 to (+?)[5]	$\frac{1}{8}$	..	$\frac{1}{4}$*[4]	..	..	
		133,333	0 to +	0	..	..	$\frac{3}{5}$ (?)	..	
Digitalin . . .	375,000	700,000	0	0	..	$\frac{2}{5}$*	..	..	Complete addition.
		466,667	$\frac{1}{4}$	$\frac{1}{4}$	..	..	$\frac{3}{5}$	..	
Picrotoxin . .	100,000	300,000	0	0	..	$\frac{1}{4}$*	..	..	Complete addition.
		200,000	0	0	..	..	$\frac{4}{6}$	..	

Saponin . . .	75,000	100,000	0 to $\frac{1}{4}$	$\frac{1}{12}$	..	$\frac{3}{4}$*	..	..	Complete addition.
		66,667	....	$\frac{2}{3}$ (?)	..	..	$\frac{2}{3}$	..	
Caffein . . .	<10,000	60,000		0	..	$\frac{0}{7}$	..	..	Doubtful; probably addition.
		40,000	0 to +	$\frac{1}{4}$	..	..	$\frac{1}{6}$	..	
Morphin . . .	450	1,000	0 to +	$\frac{1}{4}$	..	$\frac{3}{5}$*	..	..	Addition doubtful. No antagonism.
		667	0 to +	$\frac{2}{4}$	..	..	$\frac{4}{5}$	..	
Nicotin . . .	125,000	400,000		0	$\frac{1}{5}$	..	..	..	Incomplete addition.
		200,000	0 to $\frac{2}{5}$	$\frac{1}{5}$	..	$\frac{1}{4}$*	..	..	
		133,333	0 to $\frac{2}{5}$	$\frac{2}{5}$	..	..	$\frac{3}{5}$	..	
Quinin	9,500	18,000	0 to $\frac{1}{4}$	0 (?)	..	$\frac{1}{4}$*	..	..	None.
		12,000	0 to $\frac{1}{4}$	$\frac{1}{4}$ (?)	..	..	$\frac{0}{4}$ (?)	..	
		9,000	+	+	..	..	..	$\frac{4}{4}$	
Veratrin . . .	175,000	500,000	0 to $\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	..	..	..	None.
		250,000	0 to +	+	..	+	..	..	
		166,667	0 to +	+	..	..	+	..	

[1] The fractions indicate the proportion of animals which died in twenty four hours.
[2] + means that all the animals died.
[3] 0 that none died.
[4] * indicates a further fatality after twenty four hours.
[5] The observations marked with (?) are questionable

Column two shows the concentration just fatal to two-thirds of the animals, derived from my entire series of experiments.

Column three states the extremes of mortality in different series of experiments, the average of the entire series being given in column four.

Columns six to nine show the fatality when strychnin was added to the solution. The concentrations state the amount of strychnin which we supposed to be present, but the fatality for the strychnin alone indicates that the stock solution was too weak, for some unknown reason. The concentration as stated is therefore only relative. For this reason it is impossible to draw quantitative conclusions. The qualitative results are stated in column ten. They show that the strychnin modifies the toxicity of the poisons as follows:

1. The toxicity is neither greater nor less than where the poison is used alone; chloral, morphine (?), quinin, veratrin.

2. The toxicity is greater, but not as great as would correspond to the sum of the toxicity of the two poisons: potassium cyanid (?); caffein (?); nicotin.

3. The toxicity corresponds approximately to the sum of both poisons: digitalin, picrotoxin, saponin.

In no case was there any evident antagonism; *i. e.*, the strychnin never lessened the toxicity of the poison. This result was unexpected, but it applies only to the conditions of the experiment; it is quite possible that an antagonism would be observable in some cases if these conditions were varied, — if, for instance, a half or quarter fatal concentration of strychnin were added to the lethal concentration of the drug. This is indicated by some experiments on adult fish:

Two out of three fish die when placed in 1 : 200,000 strychnin, or 1 : 333 chloral. Combining the two poisons in this concentration increased the toxicity, so that all the fish died. When a third of the fatal dose of chloral was added to the 1 : 200,000 strychnin, only one fish died out of three. The chloral therefore has a distinct, although slight, saving effect in this ratio. This was not sufficient to save animals placed in 1 : 150,000 strychnin.

The uncertainty as to the strength of the strychnin precludes any conclusion as to the existence of synergism; *i. e.*, of a greater toxicity than would correspond to the sum of both poisons. This could of course occur only with digitalin, picrotoxin, and saponin; but the results speak against the probability of such an action.

Two of the drugs which give complete summation belong to the

saponin group. Experiments on ova indicate that these increase the absorption and thereby the toxicity of strychnin; and this may account for the summation. The summation in the case of picrotoxin, caffein, and nicotin may be physiologic, since these act partly on the same structures as strychnin. With the cynanid it is possible that the summation is due to asphyxia. It is interesting that there is no summation with quinin and veratrin, which probably act on different structures.

Conclusions. — Funduli respond to a large variety of poisons by a simple, progressive paralysis, with few special symptoms. When the drug is placed in the water, the course of the poisoning is quite slow when the concentration is near the fatal limit; it can be materially shortened by increasing the concentration (Table I). Nicotin and digitalin act most rapidly.

A list of fatal concentrations is furnished in Table II. The concentration necessary to kill all the fish generally exceeds by one-third that required to kill two-thirds of the animals. The toxicity is generally twice as great in distilled water as in sea water; with saponin, however, the reverse holds true, which is readily explained by the mechanism of its action. The toxicity of chloral and probably nicotin and caffein is the same in both media.

Recently hatched fish are considerably more susceptible than adults to three of the poisons, much less susceptible to four; with six poisons the toxicity is the same for both. The differences, when present, hold for both sea water and distilled water.

The result of the addition of strychnin to other poisons is shown in Table III. It was not observed to lessen their effect. With four poisons there was no addition of the toxicity; with three a partial addition occurred; three others showed complete summation of the toxic effects.

IV. The Effects of Poisons on the Developing Ova, with Especial Reference to the Embryonic Heart.

The ova were fertilized and allowed to develop for variable periods, when three to five specimens were placed in Syracuse watch-glasses with the solution to be tested, and observed at intervals until death. The poisons were those of the preceding paper; but it was soon noticed that larger doses were required and that the poisoning ran a much slower course. To this there are very few exceptions.

The action of the heart can be watched very easily, furnishing a good opportunity for the observation of the cardiac effects of these poisons. In the following, these are compared with the results obtained by Pickering[1] on the nerve-free heart of the embryonic chick. Stress of other work in the limited season prevented me from investigating the time when the nerves appear in the fundulus heart, nor could I find any data on this question. It is, of course, of fundamental importance for the interpretation of my results. The very slow action of my poisons also constitutes an important difference between my observations and those of Pickering.

The course of normal development. — The rate of development is considerably influenced by the temperature and perhaps by other conditions.

Within an hour after fertilization the blastodisc can be observed. The four-cell stage is reached in about two to three hours; eight cells in four and one-half hours; large number of cells in seven and one-half hours. The primitive streak appears in about thirty hours. In one to two days the embryo is well developed, but without pulsating heart. The heart is seen pulsating in about two and one-half days, and the vessels are visible a half-day later. The development then proceeds gradually until the time of hatching, which begins in eleven to seventeen days (mean, thirteen and one-half days). The different eggs of a single lot hatch at somewhat different times, the period of hatching extending over nearly a week. Three days after the hatching of the first eggs may be accepted as a fair average.

Summary of the results. — It seems more profitable to proceed at once to the discussion of the main results, leaving the details for the appendix, where they may be consulted.

Changes in the appearance of the embryo. — Chloral 1 : 100, and barium chloride 1 : 100, produced a *yellow discoloration of the embryos and rendered the tissues more transparent.* The blood is also partly laked. The effect occurs in one or two days. Barium chlorid in 1 : 1000 dilution does not produce this effect.

Digitalin and saponin, in fairly strong solution, also *lake* the blood, but do not cause discoloration and transparency of the tissues.

Poisons which cause rapid death of the segmenting ova (*i. e.*, before the development of the embryo) also produce *liquefaction of the protoplasm.* Potassium cyanid, digitalin, quinin, and veratrin belong to this class.

[1] J. W. Pickering: Journal of physiology, 1893, xiv, pp. 383-466.

Older ova are rendered opaque after death; *opacity* during life was observed with sodium fluorid 1 : 1000, in one day (1 : 20,000 did not produce this effect) and with quinin and perhaps caffein. Strychnin also caused opacity, but only after the heart had been so severely injured that there was no circulation.

The *quantity of blood* appears to be diminished by caffein, nicotin, and strychnin.

Effect on development. — This differs somewhat in the segmenting ova and in the embryo.

Segmentation is arrested by potassium cyanid 1 : 1000, digitalin 1 : 200,000, quinin 1 : 1000, and veratrin 1 : 1000. The effect begins in five hours with the cyanid, in twenty-three hours with the digitalin, in three to eight hours with quinin, and in eight to twenty-four hours with veratrin.

Segmentation is somewhat retarded by nicotin 1 : 10,000 and quinin 1 : 10,000. It is *not affected* by strychnin 1 : 1000 or veratrin 1 : 10,000.

Development of older embryos is retarded by barium chlorid 1 : 100, digitalin 1 : 200,000, nicotin 1 : 10,000, pilocarpin 1 : 10,000, quinin 1 : 10,000, strychnin 1 : 10,000. Short immersion in potassium cyanid 1 : 1000, also retards the development by about three days. The embryos in the digitalin and nicotin are deformed, the others not.

The following *do not cause a material retardation* of the development of the older embryos: chloral 1 : 100, barium chlorid 1 : 1000, sodium fluorid 1 : 1000, picrotoxin 1 : 1000, saponin 1 : 50,000, atropin 1 : 1000, veratrin 1 : 1000.

Hatching is often impeded, even when there is no apparent influence on the development up to this period.

It occurs *normally* in sodium fluorid 1 : 20,000, saponin 1 : 50,000 and in non-fatal concentrations of strychnin.

It is *delayed by a few days* in chloral 1 : 100 (seven day immersion, then pure sea water); potassium cyanid 1 : 1000 (short immersions) and picrotoxin 1 : 1000.

It is *delayed by several weeks* in digitalin 1 : 200,000, saponin 1 : 250,000, pilocarpin 1 : 10,000 and 1 : 1000, quinin 1 : 10,000, veratrin 1 : 10,000, and occasionally in caffein 1 : 100 and potassium cyanid 1 : 1000 (short immersion).

The other solutions cause death either at the time when the control specimens hatch or before.

Effects on the heart. — The heart of the fundulus embryos is

tubular, and consists of a pulsating sinus, auricle, and ventricle, which beat in regular sequence at a rate varying between 90 and 160 per minute, according to the specimen and according to circumstances. The influence of temperature is fairly pronounced. In the majority of cases, however, the rate is very close to 120.

Aconitin and apomorphin could not be well studied. Sodium fluorid and picrotoxin have but little effect, producing a slight weakening without the change of rate. Atropin also had but little effect, causing a moderate slowing without weakening.

All the other poisons produce very pronounced changes in the heart, of which progressive *slowing* is a prominent feature.

The poisons may be grouped under two types, the diastolic and systolic.

The *diastolic type* slows and weakens the beats from the start, the heart remaining fairly dilated and retaining a fair quantity of blood, even after stoppage. This occurs in the median position.

This group includes chloral, potassium cyanid, saponin, nicotin, and quinin.

With saponin the weakening is much more pronounced than the slowing. With quinin the heart sometimes, but rarely, becomes permanently contracted and stringy.

The *systolic type* shows at first moderate slowing, but with some increase in the force of the contractions and a distinct systolic tendency. The slowing becomes more and more pronounced, and the contractions progressively weaker. This weakening is evidently due to decrease of the diastolic expansion. The quantity of blood in the heart decreases, there being usually an accumulation behind the sinus. The heart becomes more and more contracted, until it appears finally as a weakly pulsating, bloodless cord, a condition which may be conveniently termed "stringy."

This group comprises barium chlorid, digitalin, caffein, physostigmin, pilocarpin, strychnin, and veratrin.

The changes go through the entire cycle, as described, in caffein, pilocarpin, 1 : 1000 strychnin.

With the other drugs (barium chlorid, digitalin, veratrin, and 1 : 10,000 strychnin) the changes start in the same manner, but do not go so far, the heart stopping when still fairly dilated, and containing a small quantity of blood. The later phases of physostigmin were not observed.

Three of the drugs of this group show a slight, rather doubtful

acceleration, preceding the slowing, viz.: barium chlorid, nicotin, veratrin. This quickening was so small that it might possibly be accidental. It was absent with all the other drugs of the group.

Irregularity is present in both series. It consists either in arythmia or regurgitation and reversal of the contractions.

Arythmia. — When this occurs, it is almost always produced by excessive slowing of the ventricle. (In only a very few isolated instances was the auricle observed to beat slower than the ventricle; in a very few cases it has the same rate as the ventricle, and slower than the sinus.) The ventricular slowing was present in chloral, potassium cyanid, nicotin, and quinin of the diastolic group, and in barium chlorid, strychnin, and veratrin of the systolic group. It was absent in saponin of the diastolic group, and in caffein and pilocarpin of the systolic group.

The rate of the slowed ventricle is usually quite regular, and bears a definite simple ratio to that of the auricle and sinus, — usually 1 : 2, but sometimes as slow as 1 : 10. Irregularities are seen only at the start, when the ventricle still beats at nearly the rate of the other parts of the heart, dropping an occasional beat (veratrin); and rarely and to a slight degree with barium chlorid and digitalin. With the last drug an alternating fast and slow rhythm is sometimes observed.

Regurgitation and reversal of the beats. — This is a common phenomenon with most of the poisons which produce arythmia: chloral, potassium cyanid, digitalin (rare), nicotin, quinin, strychnin, veratrin (occasionally); but it was not observed with barium chlorid. It is especially prominent with nicotin. It is sometimes due to strong contraction of the ventricle, with weak closure of the valvular portion. In other cases, however, the direction of the contraction is undoubtedly reversed. These reversed beats alternate with normal beats, often in groups. This is especially marked with strychnin, a single contraction in the right direction being followed by a number in the reversed direction, and then by a rather prolonged pause, when the cycle is repeated.

Temporary recovery from the irregularity, especially from the arythmia, is common. It was observed with barium chlorid, caffein, nicotin, strychnin, and veratrin.

The *conduction of the contraction* was visibly slowed with nicotin and quinin; *a pause between the contraction of the different parts of the heart* was observed with nicotin; *a pause after each contraction* with nicotin and strychnin.

To distinguish which actions were muscular or nervous, the changes were compared at a very early period after the development of the heart (the ova being transferred to the solutions before the heart had appeared), and at a later period. They were found to be identical in the four poisons with which this comparison was made (nicotin, quinin, strychnin, and veratrin).

Comparison of the results in fundulus embryos with those observed by Pickering on the heart of the embryonal chick. — Substantial agreement exists with chloral (chloroform), potassium cyanid (hydrocyanic acid), atropin, and caffein.

The agreement is only partial with barium chlorid, digitalin, nicotin, strychnin, and veratrin. With nicotin and strychnin Pickering observed a primary acceleration, which is absent in the fundulus. Irregularity is a prominent characteristic of the action of nicotin and veratrin on fundulus, but is not described by Pickering. A systolic standstill was observed by Pickering with digitalin, and a diastolic standstill with nicotin. Both these poisons produce a median standstill in fundulus. Strychnin, which caused a very pronounced systolic standstill with fundulus, arrested the chick's heart in diastole.

Influence of the concentration of the poison. (See also Table I.) — The course of the intoxication is generally much less affected by the concentration of the poison than is the case with adults. This is probably connected with the slow course of the poisoning; for the correspondence of concentration and effect is much closer when death occurs promptly, as with digitalin, saponin, and potassium cyanid. Distinct differences in the severity of the symptoms and in the fatality can also be made out between 1 : 1000 and 1 : 10,000 solutions of nicotin, pilocarpin, quinin, and strychnin; and with very young embryos, with veratrin. Older embryos do not show this difference in response to the two veratrin solutions. In a 1 : 1000 solution of barium chlorid, the effect starts more promptly than in the 1 : 5000 solution; but the fatality is the same. 1 : 1000 and 1 : 100 barium chlorid solutions behave alike. There is also no perceptible difference between 1 : 100 and 1 : 1000 caffein solutions.

Influence of sea water and distilled water. — The sea-water solution was distinctly more toxic than the distilled water in the case of saponin; less toxic with nicotin (very young embryos), quinin (slightly), and veratrin (somewhat doubtful). Strychnin acted identically in both media. These results correspond to those observed in hatched and adult funduli.

Influence of the age of the ova on toxicity. — In comparing the effects of the poisons on ova transferred to the solutions at various intervals after fertilization, it is seen that the toxicity is not modified between the time when the heart has appeared and the period of hatching (*i. e.*, between the second and twelfth day). Quinin is the only apparent exception to this rule, the younger embryos appearing more susceptible. A number of poisons, however, showed marked difference in the severity of their effects when the ova are exposed to them prior to the development of the embryo, *i. e.*, within an hour after fertilization. These immature ova are more susceptible than the older to digitalin, quinin, and veratrin; less susceptible to potassium cyanid and strychnin. Nicotin is about equally toxic to all ages.

The embryos are markedly more susceptible to all the poisons at the time when the hatching of the control specimens occurs. Concentrations which did not kill until this time, then became suddenly fatal. This increased susceptibility at the hatching period is most marked with chloral, barium chlorid, picrotoxin, atropin, and nicotin; it is less pronounced with digitalin, caffein, pilocarpin, quinin, strychnin, and veratrin. The experiments with sodium fluorid and potassium cyanid were not sufficiently varied to decide this point. The time required for the development of the symptoms and for death is shown by Table V.

The table shows that the *very young embryos* usually die fairly promptly — with potassium cyanid, digitalin to 1 : 100,000, and veratrin usually within twenty-four hours; with digitalin 1 : 200,000, in two to three days. Nicotin 1 : 10,000 and quinin and strychnin, 1 : 1000, do not kill these embryos, death occurring later, after five to twenty-one days, as with the older specimens.

Older embryos died very slowly in the poisons: death occurred acutely (*within one day*) only in potassium cyanid (1 : 1000), digitalin (1 : 10,000 and 1 : 100,000) and saponin, (1 : 10,000); in sodium fluorid (1 : 1000) and saponin (1 : 10,000) it occurred in one to four days.

In all the other poisons it occurred only after four to twelve days: saponin (1 : 25,000), nicotin (1 : 1000), physostigmin (1 : 1000), quinin (1 : 1000), strychnin (1 : 1000), veratrin (1 : 1000).

To appreciate thoroughly the delay of death, it must be remembered that the concentrations of these solutions are from ten to two hundred times as great as those which sufficed to kill the hatched and adult funduli within twenty-four hours.

The following concentrations are not fatal until the time of hatching approaches:

Chloral (1 : 100), barium chlorid (1 : 100), digitalin (1 : 200,000), picrotoxin (1 : 1000), atropin (1 : 1000), caffein (1 : 100), nicotin (1 : 10,000), strychnin (1 : 10,000), and veratrin (1 : 10,000).

Pilocarpin 1 : 1000 did not prove fatal, but prevented hatching, the ova surviving three or four weeks, when they died, probably a natural death.

In marked contrast to the slow death, however, is the usually prompt initiation of the effects.

Table VI. presents a rough approximation of fatal doses for fundulus ova.

Comparison of toxic doses for ova and hatched funduli. — The toxicity seems to be less for the ova than for the hatched funduli. The difference is but small (not over 1 : 3) with chloral, digitalin, and saponin. It is moderate (1 : 5 to 15) with sodium fluorid and quinin. It is very considerable (1 : 25 to 250) with barium chlorid, potassium cyanid, picrotoxin, caffein, nicotin, strychnin, and veratrin.

TABLE V.

TIME REQUIRED FOR THE DEVELOPMENT OF THE SYMPTOMS AND FOR DEATH.

A: OVA PLACED IN THE SOLUTIONS WITHIN AN HOUR AFTER FERTILIZATION.				
Poison.	Concentration. 1:	Retardation of development begins.	Development arrested.	Death and Liquefaction.
Potassium cyanid	1,000	5 hrs.	30 hrs.	2 or 3 d.
Digitalin . . .	10,000	< 3 "	....	< 3 hrs.
	100,000	5 × 24 "	....	5 × 24 hrs.
	200,000	18 "	....	2 × 3 d.
Nicotin	10,000	6 × 26 "	....	18 to 21 d.
Quinin	1,000	1½ "	3 × 8 hrs.	3 × 8 d.
Strychnin . . .	1,000	1 to 3 d.	not.	14 d.
Veratrin . . .	1,000	8 × 36 hrs.	....	16 × 48 hrs.

[1] × = The phenomenon occurred between the times joined by this sign.

TABLE V. — *Continued.*

B: Older Embryos.

Poison.	Concentration. 1:	First effect noticed on heart.	Heart rate fallen below 60 per min.	Irregularity of heart appears.	Other phenomena.	Death.
Chloral	100	5 × 20 hrs.[1]	6 d.	1 × 3 d.	Laking and transparent 2 × 3 d.	5 to 10 d.°[2]
Barium chlorid	100 and 1000	1½ hrs.	Not till death.	3 to 6 d.	Laking and transparent 1 × 2 d.	7 to 16 d.°
	5,000	7 d.				
Sodium fluorid	1,000	1 to 3 d.	" " "	....	Opacity about 1 d.	2 to 4 d.
Potassium cyanid	1,000	< 1 hr.	1 hr.	....	....	4 × 21 hrs.
Digitalin	10,000	½ × 1½ hrs.	....	....	....	½ × 1½ hrs.
	100,000	1½ × 3 hrs.	....	< 3 hrs.	....	3 × 18 hrs.
	200,000	Not till death	....	....	....	1 to 17 d.°
Picrotoxin	1,000	" " "	....	....	....	8 to 13 d.°
Saponin	1,000	1 to 2 hrs.	....	....	....	1 to 4 hrs.
	10,000	1¼ × 20 hrs.	....	....	....	2 to 3 d.
	25,000	1 × 2 d.	....	....	....	2 to 6 d.
	50,000	None.	....	....	....	Not fatal.
Atropin	1,000	....	....	....	....	9 to 15 d.°
Caffein	100 and 1000	3 hrs. to 11 d.	....	....	....	11 to 24 d.°
Nicotin	1,000	....	1 d.	< 20 hrs.	....	2 to 7 d.
	10,000	3 to 8 hrs.	3 to 6 d.	4 to 7 d.	....	9 to 20 d.°
Physostigmin	1,000	5½ hrs.	1 d.	....	....	5 to 9 d.
Pilocarpin	1,000	2 d.	2 d. before death	....	....	3 to 4 w.[3]
Quinin	1,000	3 × 21 hrs.	1 to 3 d.	....	....	4 to 8 d.
Strychnin	1,000	< ½ hr.	1 d.	....	Stringy in 5 to 8 d.	4 to 12 d.
	10,000	1 × 2 d.	8 × 11 d.	....	Not stringy	11 to 14 d.°
Veratrin	1,000	1 hr. to 2 d.	6 to 8 d.	4 hrs. to 2 d.	....	10 d.
	10,000	1 hr. to 8 d.	5 to 9 d.	5 to 9 d.	....	11 d.°

[1] × = The phenomenon occurred between the times joined by this sign.
[2] ° = Death occurred only at the period of hatching.
[3] Natural death of unhatched embryos.

Replacement in pure sea water. — When the ova are exposed to the poison for varying periods, and are then replaced in pure sea water, recovery does not generally occur, but the poisoning proceeds almost as if the ova had been left in the solution. This (absence of recov-

TABLE VI.

FATAL CONCENTRATION OF POISONS FOR FUNDULUS OVA (SEA WATER, EXCEPT BARIUM CHLORID AND SODIUM FLUORID).

Drug.	Newly fertilized ova: 1:	Before hatching: 1:	At time of hatching: 1:
Chloral	....	<100	>100
Barium chlorid	....	<100	>5,000
Sodium fluorid	....	1,000 × 20,000	20,000
Potassium cyanid	1,000	1,000 × 10,000	
Digitalin	200,000	100,000 × 200,000	>300,000
Picrotoxin	....	<1,000	>1,000
Saponin	....	25,000 × 50,000	25,000 × 50,000
Atropin	....	<1,000	>1,000
Caffein	....	<100	>1,000
Nicotin	<10,000	1,000 × 10,000	>10,000
Physostigmin	....	>1,000	>1,000
Pilocarpin	....	<1,000	<1,000
Quinin	<1,000	1,000 × 10,000	5,000 × 10,000
Strychnin	<1,000	1,000 × 10,000	10,000 × 100,000
Veratrin	1,000	1,000 × 10,000	10,000 × 100,000

ery) was noted in the following solution, the ova having been exposed to the poison for the time noted: potassium cyanid 1 : 1000, one day; digitalin 1 : 10,000, one and one-half hours; 1 : 100,000, twenty-four hours; picrotoxin 1 : 1000, five and twelve days; atropin 1 : 1000, six and twelve days; nicotin 1 : 10,000, to within a few days of death; pilocarpin 1 : 1000 and 1 : 10,000, two or three weeks; strychnin 1 : 1000, nine days; veratrin 1 : 1000, ten days.

Only the following showed some recovery: potassium cyanid 1 : 1000, very young, to third day; older, just after stoppage of

heart; chloral 1 : 100, seven days, death is prevented, but discoloration persists.

Discussion of the cause of the high resistance of the ova to poisons. — The slow course of the symptoms and the greater concentrations required to produce death indicate a relatively very high resistance power, which is perhaps the most interesting general phenomenon of this research. This could be accounted for either by a true insusceptibility of the protoplasm or by relative impermeability of the egg-shells.

There is no evidence whatever of the first, of the *insusceptibility of the protoplasm*. It can only play a minor rôle, at most. The fact that the resistance exists for poisons of very different actions speaks strongly against a histogenetic tolerance. The sudden and very marked increase of toxicity at the moment of hatching is a still stronger argument against this species of tolerance; it is almost inconceivable that the eruption of the embryo from the shell should radically alter the response of its protoplasm to poisons.

This also disposes of the possibility that the low toxicity could be referred to *habituation*, occurring as a result of the slow course of the poisoning. As has been said, the toxicity does not at all diminish with the time of exposure, but invariably increases as the time for hatching approaches. Embryos which have been reared and hatched from ova kept in the following dilute solutions, die very promptly in these solutions as soon as they have left the shell:

Sodium fluorid (1 : 20,000); digitalin (1 : 200,000 to 1 : 300,000); saponin (1 : 50,000); caffein (1 : 100); pilocarpin (1 : 10,000); strychnin (1 : 100,000); veratrin (1 : 100,000). The susceptibility of the hatched fish does not appear to be diminished in the least by their having been reared in the poisons.

There remains but one possibility, a *relative impermeability* of the ova to the poisons. The slow course of the poisoning and the relatively low toxicity can be readily understood by making this assumption. Since the embryos exhibit characteristic symptoms, starting generally within a day and often within an hour after the exhibition of the poison, the impermeability cannot, of course, be absolute.

The theory that the poisons penetrate but slowly, and that this slow penetration is responsible for the slow action and the low toxicity, agrees closely with all the observed phenomena.

Saponin and digitalin, which are known to increase the permeability of cell membranes, both act very promptly, and their toxicity

is nearly equal for ova and adults. Chloral, which also has a "laking" action, also has nearly the same toxicity for ova and adults. Cyanid, which may be assumed to penetrate fairly readily in virtue of its volatility, is also promptly effective.

The influence of the concentration of the poisons also favors this view. If the insusceptibility were histogenetic, the concentration of the poison should have a very marked effect, whereas the concentration would be much less important if the penetration of the poison is very slow. As we have seen, the course of the poisoning in ova is not very conspicuously affected by relatively large differences in concentration, except with digitalin, saponin, and potassium cyanid, for which there is reason to assume a more ready penetration.

The increased toxicity as the time for hatching approaches is readily explained, either by assuming chemical changes in the egg-shell at this period, increasing its permeability, or by actual ruptures of the shell. Neither of these phenomena was directly demonstrated, but their assumption seems quite natural.

Since the outer, tough egg-shell is the one which is broken in hatching, it appears that this is the membrane which offers the barrier to penetration.

The greater susceptibility of the ova at the earliest stages of development to digitalin, quinin, and veratrin, and the lesser susceptibility to potassium cyanid and strychnin, can scarcely be explained by differences in permeability, or they would be more uniform. They are probably functional.

The most serious difficulty to the impermeability theory of the tolerance is offered by the behavior of the embryos which have been exposed for some days to the poison and are then transferred to pure sea water. No recovery occurs, as a rule, the poisoning proceeding as if the ova had been left in the solution. This can be explained partly by assuming that the injuries have already progressed beyond recovery at the time when the transfer is made; but this explanation is not adequate in the case of picrotoxin, where the symptoms are very slight at the time of transfer, but where death occurs, several days later, at the time of hatching. This makes it necessary to assume that this is a rather critical period, which cannot be successfully passed even when the embryo has been slightly injured.

If the slow penetration is the main cause of the tolerance, it should be possible to increase the effects by increasing the permeability of the ovum. This was tried by adding saponin (1 : 25,000 and

1 : 50,000) to strychnin (1 : 10,000). This resulted in the phenomena usually produced only by a 1 : 1000 solution of strychnin, both as to time and quality of the symptoms. (It should be recalled that the cardiac effects of saponin and strychnin are entirely distinct, so that the effect is not due to the addition of the actions of both poisons, but to a true increase of the strychnin effect.)

The thought suggested itself, that the non-penetration of the poison could be due to insolubility of the alkaloidal salts in the lipoids which might be present in the egg-shell, as described by Overton for cell-membranes. The low toxicity of picrotoxin rendered this theory *a priori* improbable. To test it, sufficient sodium hydrate was added to distilled water solutions of the salts of strychnin, quinin, veratrin, and nicotin, to liberate the alkaloids. The toxicity was not materially increased; in the case of strychnin it was even diminished, through the precipitation of the alkaloid. It would seem, therefore, that the relative impermeability of the egg-shell is not due to the presence of lipoids.

The impermeability of the fundulus ova was at first rather surprising, since a very high permeability of the membrane had been generally assumed, in order to account for the slight osmotic phenomena exhibited by these forms. It is evident, however, that this could be equally well explained by assuming a very slight permeability to both water and salts. Whilst I was doing this work, O. H. Brown[1] succeeded in demonstrating the relative impermeability of the fundulus ova to salts by physical means.

Conclusions. — The development of early embryos is readily arrested by a number of poisons, but that of older embryos is not generally affected. Hatching, however, is often indefinitely delayed.

The effects of poisons on the embryonic heart consist generally in slowing, of the whole heart, or especially of the ventricle. Two types of slowing may be distinguished: diastolic (chloral, potassium cyanid, saponin, nicotin, and quinin) and systolic (barium chlorid, digitalin, caffein, physostigmin, pilocarpin, strychnin, and veratrin). Both types may exhibit a relatively greater slowing of the ventricle, which then beats in a simple ratio to the auricle and sinus, generally at one-half the rate. Reversal of the direction of the contraction is also a common phenomenon, especially with nicotin. The effects are probably muscular rather than nervous. The details are given in the appendix.

[1] O. H. Brown: This journal, 1905, xiv, p. 354.

The results differ considerably in detail from those described by Pickering for the heart of the embryonic chick, although there is a general agreement.

The concentration of the poison has a comparatively slight influence on the effects, within wide limits.

The differences between solutions in sea water and distilled water are similar to those observed with adults.

The toxicity may vary with a number of poisons before and after the appearance of the embryo. From the time when the heart appears until the approach of the hatching period, the toxicity remains constant. It is considerably increased at the time when the control specimens hatch.

The course of the intoxication is generally very much slower, and the toxicity very much less, than with adults. This is due to the low permeability of the egg-shell for poisons. Drugs which penetrate readily are nearly as active as in adults. The penetration can be increased by saponin. Free alkaloids are not absorbed more readily than their salts.

V. Appendix. — Details of the Effects on Ova.

Details of the observations. — These will only be given summarily, since the actual protocols would be needlessly tedious. It should be remembered, however, that these short summaries are condensed from a very considerable number of experiments. Each series consists of three to six eggs.

Chloral in sea water. — 1 : 100, four series.

General effects. — The embryos became transparent and yellow, and the blood laked, within three days. They move freely in the shell after six days.

Development. — This is not visibly affected, but the hatching is delayed by three to nine days if the ova are removed to sea water after seven days' sojourn in the chloral solution. Those which were left in the solution died before the period of hatching.

Heart. — No effect is produced up to five hours.

In twenty-four hours there is a slight slowing, to about 90, but the contractions are of normal type.

On the third day the slowing has advanced to about 80, the beats are slightly irregular, and the heart is dilated. The irregularity consists in the occasional loss of some beats; the ventricle may beat at

half the rate of the auricle and sinus; the beat of the ventricle may be reversed. The heart is generally dilated and somewhat weakened.

In six days the heart has slowed to 60, it is weaker and much less filled. The irregularity is not so pronounced. The circulation through the vessels is slow.

In seven days the slowing has progressed to 30 or 40 very weak beats, the heart being moderately filled. It contains some blood after death.

These effects correspond fairly with the observations of Pickering (p. 445) on chloroform: he found a very slight slowing with exaggerated diastole; the rhythm then became somewhat irregular. The ventricle then beat at the rate of 90, the auricle less. A reversed rhythm of 140 was then observed, alternating with the same rhythm in the normal direction. Finally, the auricle and ventricle both stopped in extreme diastole, the auricle first.

Age. — No difference exists between embryos 2 and 12 days old. Older embryos die about the time when hatching would naturally occur.

Time. — The first effects (cardiac slowing) are noticeable in five to twenty hours. The laking, transparency, and discoloration occur between the second and third days. Death occurs after five to ten days.

Recovery in sea water. — Replacement in sea water after seven days' sojourn in the chloral solution does not remove the transparency and yellow discoloration; but it arrests the toxicity, so that the embryos survive and hatch after four to ten days.

Barium chlorid in distilled water. — 1 : 100, one series; 1 : 1000, three series; 1 : 5000, one series.

General effects. — 1 : 100 produces the same effects as chloral: the embryo becomes yellow and translucent on the second day. This phenomenon is absent with the more dilute solutions.

Development. — The 1 : 100 solution retards the development. The others proceed normally to hatching, but die before hatching occurs. Rupture and protrusion through the shell occurred in only one ovum.

Heart. — A moderate slowing (to about 80 beats) occurs within two hours, the beats being strengthened and the systolic force and duration increased. This condition may persist until the last day before death, the heart being merely weakened, but remaining filled with blood; or it may be replaced in three to thirteen days by irregu-

larity of the ventricle, which generally beats regularly at half the rate of the auricle and sinus. Other types of irregularity are seen occasionally: in one case a rather prolonged pause occurred between the contraction of the auricle and ventricle, with a rate of 50 beats; in another case the auricle beat at half the rate of the ventricle; in another the sinus and auricle beat regularly at 100, the ventricle irregularly at 30 to 50. The irregularities may disappear and reappear.

As poisoning advances, the beats grow weaker and the circulation less. The heart generally retains a fair amount of blood even after death.

This description differs markedly from that of Pickering (p. 422), who observed a marked simple depression, culminating in diastolic standstill.

Age. — This has no effect until the time of hatching approaches, when the embryos die. Older embryos therefore die in a shorter time than the younger specimens.

Time. — The first effects are noted in about one and one-half hours, with 1 : 100 and 1 : 1000; in seven days with 1 : 5000. The irregularity appears in about two days. Death occurs in seven to sixteen days, about the time when hatching is due.

Dose. — The time of death is the same for the three dilutions; the poisoning follows the same course in 1 : 100 and 1 : 1000, but with 1 : 5000 the appearance of the symptoms is much delayed.

Sodium fluorid in distilled water. — 1 : 1000, two series; 1 : 20,000, one series.

General effects. — 1 : 20,000 has no action until after the embryos are hatched, when they die promptly. In the stronger solution there is some opacity in one day. These die two to four days before the time for hatching.

Heart. — This is somewhat weakened without change of rate.

Age. — There is no difference between the five and eight day ova.

Time. — The first symptom, opacity, occurs in one day; the cardiac weakening is pronounced in about two days. Death occurs in two to four days.

Potassium cyanid in sea water. — 1 : 1000, six series; 1 : 10,000 and 1 : 100,000, each one series.

General effects. — With young ova (*i. e.*, before the development of the embryo) these consist in arrest of development, beginning in about five hours. The embryos remain apparently lifeless for two or three days, during which they may be revived (see following paper).

The protoplasm then liquefies. When the embryo has developed, liquefaction also occurs, but more slowly — in some in twenty-two hours, in all in three days. Recovery is possible after stoppage of the heart, but not after liquefaction.

Heart. — Within one and one-fourth hours this is much slowed (to 24 or 28 beats) and very weak. The condition is the same in three hours. In seven hours the heart is arrested. If placed in pure salt water, recovery begins within an hour, the heart being at first often irregular, with the ventricle at half the rate of the auricle and sinus, and with regurgitation. After a day the heart is again perfectly normal.

This agrees sufficiently with Pickering's experiments (p. 421). His effects consisted in slowing (to 50 beats) with strong regular beats, the systole being apparently increased and the diastole diminished. This was succeeded by a reversed rhythm at the rate of forty. The contraction of the auricle and ventricle was apparently simultaneous. The heart then beat slowly and weakly, sometimes in one direction, sometimes in the other, the ventricle being slower than the auricle. The standstill was diastolic.

Age. — The very young ova die less promptly and can be resuscitated more readily. This change of susceptibility occurs abruptly at the time when the vessels are developed.

Time. — With the young ova the first effect on development is noticeable in five hours; with the older the effect on the heart can be discerned in one hour. Complete arrest of development occurs in twenty to thirty hours, stoppage of the heart in six hours. Resuscitation is possible after the third day, up to the development of vessels; in some of the older embryos it is not possible after twenty-one hours.

Dose. — This is quite marked: 1 : 10,000 and below had no effect; a 1 : 1000 solution which had become somewhat weaker by standing, allowed complete development, although this was somewhat delayed and a partial fatality occurred.

Digitalin in sea water. — 1 : 10,000, three series; 1 : 50,000, one series; 1 : 100,000, two series; 1 : 200,000, four series; 1 : 300,000 two series.

General effects. — When used on very recently fertilized ova, it causes death and liquefaction. The cell division is not affected until the poisoning is well advanced (twenty-three hours); from this time on the development is greatly retarded.

Older embryos continue to develop in 1 : 300,000 and 1 : 200,000 solutions, but hatching is generally much delayed (to twenty-seven days beyond the control). The hatched embryos may be normal and live indefinitely; but they are often imperfect and deformed, and die rather promptly of paralysis, the heart being slowed and weakened, but filled.

Heart. — The first effects consist in a slight slowing (to 80) with systolic tendency. A few cases show quickening, with alternating slow and fast rhythm. The heart is then weakened, but well

TABLE VII.

TIME AFTER FERTILIZATION WHEN OVA WERE PLACED IN THE SOLUTION.

Concentration of digitalin.	Within five hours.	Three to six days.	Eight days.
1 : 10,000	First effect less than 3 hrs.	½ to 1½ hrs.	....
	Death less than 3 hrs.	½ to 1½ hrs.	....
1 : 100,000	First effect 5 to 24 hrs.	1½ to 3 hrs.	....
	Death 5 to 24 hrs.	3 to 18 hrs.	....
1 : 200,000	First effect 18 hrs.	No effect	1 to 17 d.
	Death 2 to 3 d.		
1 : 300,000	Death 3 to 5 d.	No effect.	12 to 16 d.

filled. The rate remains about constant, and the contractions are generally normal. All parts of the heart now beat at the same rate, but the ventricle may miss an occasional beat. Regurgitation is rare. The weakening then progresses, so that the circulation stops. The heart may beat at the rate of 60 to 90, somewhat irregularly. It remains well filled with blood, which may appear laked; but the embryo shows no sign of liquefaction or decoloration.

The effects differ considerably from those of Pickering (p. 407), who found digitalin and strophanthin to produce progressive slowing with some irregularity, the ventricle beating much slower than the auricle, and stopping in systole, whereas the auricle was arrested in diastole.

Age. — This has no marked influence except with the just fatal dose (1 : 200,000). This was not fatal to embryos three to six days old, whereas the younger ova die in three days, the eight-day em-

bryos in one to sixteen days. With just fatal doses the toxicity seems to be somewhat (but only slightly) increased at the time of hatching.

Dose. — This has a conspicuous effect, both on the time and fatality, as shown in the Table VII.

The *fatal concentration* lies about 1 : 200,000.

Time. — This is shown in the preceding table. The poisoning proceeds rapidly; the first effects occur within two hours (except in the case of very dilute solutions with very young embryos). The time of death varies with the dilution, from one-half hour to seventeen days.

Recovery in sea water. — This does not occur when the sojourn is extended over one and one-half hours in 1 : 10,000, or twenty-four hours in 1 : 100,000.

Picrotoxin in sea water. — 1 : 10,000, three series.

General effects. — The picrotoxin is apparently almost without action. The heart is slightly slowed, and shows exceptionally a variable rhythm. There is practically no effect on development, but hatching is prevented. It appears as if the poison became effective only when the embryo is about to hatch; death always occurring at this period, no matter when the eggs were placed in the solution.

Age of eggs when placed in solution.	Time elapsing before death.	Age at time of death.	Age at which the controls hatch.	Death occurred x days after the controls hatched.
2 days.	18 days.	21 to 23 days.	17 days.	$x = 4$ to 6 days.
8 days.	11 to 16 days.	19 to 20 days.	15 days.	$x = 4$ to 5 days.
15 days.	8 days.	21 to 24 days.	20 days.	$x = 2$ days.

The phenomenon creates the impression that the shell of the ovum is totally impervious to picrotoxin, so that the poison becomes effective only when the shell is broken. This explanation, however, is disproven by the fact that the embryos died at the same time, if they were transferred to pure sea water three to six days before death. This indicates that the poison must have been stored in the ova.

Saponin in sea water. — 1 : 1000, two series, 1 : 10,000, one series, 1 : 25,000, two series; 1 : 50,000 and 1 : 100,000, each one series. **In distilled water.** — 1 : 25,000 and 1 : 100,000, each one series.

General effects. — The blood is apparently laked, but the embryo is not discolored as with chloral. After death the amniotic sac shrinks very markedly in specimens which were in the sea-water solution.

Development. — This is not visibly affected, but the hatching is generally delayed, sometimes by four weeks.

Heart. — The effect consists in great weakening of the contractions, with moderate slowing. The heart retains a fair amount of blood.

Age. — There is no material difference in the effects on embryos which were placed in the solution two and twelve days after fertilization. The toxicity is not markedly increased just prior to hatching. Embryos which have been hatched in dilute saponin are about as susceptible to the poison as embryos hatched in pure sea water.

Time. — With just fatal doses the first effects appear in about a day, with higher concentrations in a much shorter time. The course of the poisoning is rapid. Death occurs in (sea water):

Concentration.	Time of death.
1 : 1,000.	1 to 4 hours.
1 : 10,000.	2 days.
1 : 25,000.	3 days.

Dose. — The effects are very much influenced by the dose, as shown by the preceding table. The fatal concentration in sea water lies between 1 : 50,000 and 1 : 25,000; in distilled water it is 1 : < 25,000. The greater toxicity in sea water agrees with the results on hatched and adult funduli. Sublethal doses have no effect beyond the delay of hatching.

Atropin in sea water. — 1 : 1000, three series.

General effects. — There is practically no effect until the period of hatching approaches, when death occurs. Up to this time the development is normal. Replacement in sea water does not prevent the death, the results being similar to picrotoxin.

Heart. — This is somewhat slowed (to 80 or 90) and slightly weakened.

Pickering (p. 452) observed also a fair slowing (almost to 64) with normal rhythm.

Aconitin in sea water. — The sample of the alkaloid (Schuchardt) failed to dissolve to any considerable degree in sea water, even after the addition of some hydrochloric acid. This weak solution was used in two series.

Development. — This did not appear to be affected, but hatching was generally prevented.

Heart. — This showed but little effect; only one specimen exhibited some abnormalities.

Apomorphin in sea water. — 1 : 1000, one series.

There was no effect in one day. Extensive precipitation precluded further observations.

Caffein-sodium benzoate in sea water.— 1 : 1000, one series. **In distilled water.**— 1 : 100, one series.

General effects. — The protoplasm appears to become somewhat opaque, and the amount of blood is apparently diminished. Hatching is often delayed, but may occur normally.

Heart. — In a 1 : 1000 solution, the heart remains normal for six days, but on the eleventh day it has been slowed to 60, and is contracted into an almost empty cord containing a small bolus of blood.

In the 1 : 100 solution the heart appears somewhat quickened in three hours.

In twenty-four hours it has been slowed to 70, with strong contractions and a systolic tendency.

In two days the rate is again increased from about 80 to 120, but the heart has contracted into an almost empty cord, beating weakly and somewhat irregularly.

In four to fifteen days the conditions remain nearly constant.

These actions agree sufficiently with those observed by Pickering (p. 396). He observed a primary acceleration from 88 to 100 beats. This was followed by increased tonus with great slowing (to 12 or 24 beats), and this by systolic standstill. The latter may at first be temporary with spontaneous recovery.

Time. — The effects begin in three hours with 1 : 100; 1 : 1000 required several days to produce any noticeable results. Death occurs in eleven to twenty-four days.

Dose. — The effects start more promptly in the stronger solution, but death is not hastened.

Embryos which have been raised in the 1 : 100 solution (twenty-two days) die promptly in the same solution on hatching.

Nicotin in sea water. — 1 : 10,000, five series; 1 : 1,000, one series. **In distilled water.** — 1 : 10,000, two series; 1 : 1,000, one series.

General effects. — Development is considerably retarded at all stages, and the embryos are abnormal and imperfect. The quantity of blood is decreased. Hatching is prevented, the nicotin being fatal either before or during hatching.

Heart. — The main effects consist in slowing, weakening, and irregularity. The first observable effect was slowing, to about 80; the heart is also slightly weaker, but still fairly strong. The systole and

diastole are about equal. The slowing appears to be due to the slower conduction.

When the rate has fallen to about 60, irregularity generally makes its appearance. This consists ordinarily in regurgitation. In some cases this is plainly due to very strong contractions of the ventricle, with imperfect closure of the auriculo-ventricular ring. In others the direction of the contraction is actually reversed in alternate beats, one contraction being in the right and the next in the wrong direction. The ventricle may beat at one-half to one-tenth the rate of the sinus and auricle, but generally in a simple ratio. In other cases the ventricle may have stopped altogether, while the sinus and auricle are still beating regularly, or with a long pause after every three or four beats. These pauses may also appear when the ventricle is still beating. In a few instances the ventricle continued to beat stronger than the sinus or auricle. The earlier irregularities may disappear for a time, and then reappear.

The heart gradually becomes empty, but is always fairly dilated, and never contracted into a cord, as with caffein. The rate falls to 24, while the irregularities increase. Finally, the heart stops in a median position, usually retaining some blood.

It is worthy of remark that these symptoms are very prolonged before death occurs.

In several of these series the embryos were placed in the nicotin before the appearance of the heart. In these cases the slowing and regurgitation were visible as soon as the heart developed. This indicates that these effects are muscular rather than nervous. The arythmia of the auricle and ventricle did not appear until later, so that its cause cannot be stated.

Age. — This has no perceptible influence on the toxicity, the appearance of the symptoms, or the time of death, except that the embryos which have developed up to the point of hatching die at once when the shell breaks.

Dose. — 1 : 1000 acts more promptly than 1 : 10,000.

Time. — Effects occur as follows:

Concentration.	First Effects.	Slowing below 55.	Death.
1 : 1000.	....	1 day.	2 to 7 days.
1 : 10,000.	3 to 8 hours.	3 to 6 days.	to 20 days.

Distilled and sea water. — In the specimens which were placed in the solutions forty-five minutes after fertilization, the distilled-water

solution seems distinctly more toxic, death occurring in one day in the distilled-water solution, and in three to four days in the sea water. With older ova this difference was not apparent.

Replacement in pure sea water caused no recovery in the two series in which it was tried; but both were within a very few days of death when they were transferred.

Physostigmin in distilled water. — 1 : 1000, one series.

A slight slowing was observed in five and one-half hours. This had progressed in twenty-three hours to the rate of 60, with strong regular contractions and with a systolic tendency. The conditions remained unaltered on the third day. On the fifth day two of the specimens were slowed to 20, the heart being still filled with blood. On the ninth day all the ova were dead.

Pilocarpin in sea water. — 1 : 1000, three series, 1 : 10,000, one series.

Development. — This is slightly retarded. Hatching is often interfered with, but the embryos are normal when it occurs. In case it is prevented, the ova remain alive for several weeks after the ordinary time of hatching.

Heart. — This is slowed to 80, rather weak, with systolic tendency. Later, it tends to become stringy. Irregularities are rare, and consist in inequalities of rate. Regurgitation was never seen.

Age. — There is no difference in the effects on embryos two and fifteen days old when placed in the solution. The pilocarpin is perhaps somewhat more toxic at the time when the control specimens hatch. The hatched embryos die promptly in 1 : 10,000 solution.

Time. — In a 1 : 10,000 solution the first effect is noticed in two days. The symptoms do not become serious until about two days before death. Death is probably not due to the pilocarpin, since it occurs only in three to four weeks.

Dose. — 1 : 1000 is more effective than 1 : 10,000, but is still not fatal.

Replacement in pure sea water after one or two weeks in the pilocarpin solution does not prevent interference with hatching.

Quinin in sea water — 1 : 1000, five series; 1 : 10,000, two series. **In distilled water.** — 1 : 1000, two series; 1 : 5,000, one series; 1 : 10,000, two series.

General effects. — The entire protoplasm becomes whitish and less transparent after a few days, but the ova may live for some time after the opacity has appeared. The 1 : 1000 solution causes liquefaction of recently fertilized ova within a few hours.

Development. — This is considerably delayed, especially with the younger embryos. Even the 1 : 10,000 solution produces this effect, and is apt to interfere with hatching.

Heart. — The first effects consist in slowing, the beats remaining regular and of undiminished strength. The slowing becomes gradually more pronounced, and the beats weaker; the heart, however, remains usually well filled, and is but rarely "stringy." The conduction of contraction wave is markedly slowed. The heart often remains regular, but more commonly various types of irregularity appear. The ventricle is often slower than the auricle; it may pump in the reverse direction; it is also often stronger than the auricle. In embryos which were put in the solution before the development of the heart, the rate is slowed within three days after the appearance of this organ.

Age. — The effects appear more promptly in the younger specimens, especially in those poisoned very soon after fertilization. The time of death varies correspondingly, but the fatal concentration is not markedly affected by the age. The toxicity is slightly increased at the time of hatching, but this increase is not very marked.

Time. — In the concentration of 1 : 1000 the effects occur as follows:

Age of ovum when placed in solution.	First symptoms.	Heart slowed below 55.	Death.
Less 1 hour.	3 hours.	....	3 to 8 hours.
2 to 3 days.	1½ hours.	1 to 3 days.	2 to 5 days.
8 to 12 days	3 to 21 hours.	1 to 3 days.	4 to 8 days.

Dose. — The difference between 1 : 10,000 and 1 : 1000 is very marked. The former causes only a slight retardation of the early development, and interferes with the hatching. It does not produce death, nor the cardiac effects which are characteristic of the stronger concentration.

The distilled water solution is somewhat more toxic than that in sea water.

Strychnin in sea water. — 1 : 1000, eight series; 1 : 10,000, three series; 1 : 100,000 and 1 : 200,000, each one series. **In distilled water.** — 1 : 1000, four series; 1 : 10,000, 1 : 100,000, and 1 : 200,000, each one series.

General effects. — Strychnin produces some opacity, but only after the heart has been severely injured. The formation of blood seems to be lessened.

Development. — This is not hindered to the acardiac stage (twenty-seven hours); it is then slightly delayed, but not by more than twelve hours. There is generally considerable interfering with hatching.

Heart. — A moderate slowing (80) is perceptible in one-half hour in 1 : 1000 solution, in one day in 1 : 10,000. The beats are regular, strong, with quick systole, somewhat slower diastole, and long pause. The slowing progresses to 60, the beats becoming somewhat weaker. At this time irregularity may set in, although it is often absent. The ventricle generally beats at half the rate of the sinus and auricle. The beats may occur in groups, separated by a long pause. The direction of the contraction is often reversed, the normal beat being followed by a reversed contraction; or there may be one normal beat, then several reversed, then a long pause, and then a repetition of the phenomena. Temporary improvement may occur. The rate at this time is about 40 or 50; the heart is well filled with blood, but generally weak. This stage is reached in about three days in the 1 : 1000 solution, in nine days in the 1 : 10,000. The latter concentration does not produce any further effect. In the 1 : 1000 solution, however, the changes proceed. The blood accumulates back of the sinus, the heart becoming more and more empty and contracted, so that in five days it appears as a thin, colorless cord, pulsating weakly at the rate of 24, or even 10, beats per minute. The contractions cease successively in the ventricle, auricle, and sinus.

Embryos which had been placed in the solution before the appearance of the heart, showed a slowing on the first day after the heart becomes developed, and the irregularity on the third day. The effects are therefore probably muscular.

The 1 : 100,000 solution is devoid of any effect on the heart.

These results differ considerably from those of Pickering (p. 457). He observed a primary quickening (which was never seen in the funduli), followed by slight slowing. This was succeeded by some irregularity and weakening of the systole. Death occurred in diastole.

Age. — In the very young embryos the action appears to be delayed by about the time required for the development of the heart. It would seem, therefore, that the poison begins its action only when the heart is developed. The toxicity appears to increase about the time of hatching (as with picrotoxin), for in the 1 : 10,000 solution the time of death coincides precisely with the hatching of the control

specimens. Some of the embryos, however, hatch in the 1 : 10,000, and especially in the 1 : 100,000 solutions, dying promptly after hatching (the fatal concentration for hatched embryos being 1 : 225,000).

Time. — The effects start promptly, but the course is very slow, as is shown by the following table:

Age of ovum when placed in solution.	Concentration of strychnin.	First effects.	Rate below 55.	Heart stringy.	Death.
To 1 hr.	1 : 1000.	1 to 3 d.	2 to 3 d.	4 to 9 d.	14 d.
3 to 12 d.	1 : 1000	Less ½ hr.	1 d.	5 to 8 d.	9 to 12 d.
19 d.	1 : 1000	....	1 d.	....	4 to 5 d.
7 to 9 d.	1 : 10,000.	1 to 2 d.	8 to 11 d.	Not.	11 to 14 d.

Dose. — The 1 : 100,000 solution has no effect; 1 : 1000 acts much more quickly than the 1 : 10,000, and with the latter the heart does not proceed to the stringy stage, perhaps because the time of hatching arrived and the embryos consequently died before this stage had a chance to develop.

There is no material difference between sea water and distilled water.

Replacement in pure sea water after the ova had sojourned nine days in strychnin solution does not prevent the progress of the symptoms.

The rate had fallen to 40, but the heart was well filled, at the time the transfer was made. This progressed to the stringy stage and death, just as it would have done had the ova been left in the strychnin.

Veratrin in sea water. — 1 : 1000, five series; 1 : 10,000, two series; 1 : 100,000, one series. **In distilled water.** — 1 : 1000, 1 : 5000, 1 : 10,000, and 1 : 100,000, each one series.

Development. — Embryos placed in 1 : 1000 solutions within an hour after fertilization show no retardation of development up to eight or twenty-four hours, when they have reached the many-celled stage, or even that of acardiac embryos. Then development stops, and the embryos are liquefied within one or two days. In 1 : 10,000 solution these ova develop normally to the time of hatching; the latter, however, is prevented, death occurring about this time. Older embryos (two days and upwards) are not liquefied even by the 1 : 1000 solution, death occurring, as with the 1 : 10,000 solution, about the time of hatching.

1 : 100,000 has but little effect, although it may prevent hatching in some cases.

Heart. — There seems to be a primary acceleration of 10 or 20 beats, occurring within an hour. This is followed in four hours by a slight slowing (rate of 90 to 110). The sinus and auricle preserve this rate for about five days; the ventricular beats, however, become irregular within one or two days. At first the ventricle misses an occasional beat, perhaps one in ten; this slowing of the ventricle then becomes more pronounced and regular, its rate being generally one-half, sometimes one-third or even one-tenth, that of the sinus, always in a simple, regular ratio. The auricle generally has the same rate as the sinus, but it sometimes assumes that of the ventricle. Reversal of the direction of the contractions is also sometimes witnessed. The embryos are not all affected alike. In the same lot of eggs one may be irregular in four hours, another only in nine days. It would seem that temporary recovery may occur, although I cannot affirm this with certainty. The heart is strong and well filled throughout this stage, which continues to the sixth or eighth day. The contractions then become slowed to the rate of ten, very weak and ineffective, but the heart still contains some blood. Finally, stoppage occurs, first in the ventricle, then in the auricle, then in the sinus.

In embryos which were placed in the solution before the appearance of the heart, the irregularity is just as pronounced within three days after the development of this organ, so that the irregularity is probably of muscular origin.

Pickering (p. 440) did not observe the irregularity, which is the most characteristic feature of the action on funduli. He found a slight primary acceleration, followed by considerable slowing, with a long and weak systole and complete diastole.

Age. — As has been stated, the undeveloped ova are much more susceptible to the 1 : 1000 solution. The fatality is also markedly increased at the moment of hatching. Embryos which have hatched in the 1 : 100,000 solution die promptly in the same solution.

Time. — On the developed ova the first effects appear in from one hour to two days; the arythmia in one hour to three days; the heart becomes very weak in six to eight days; death occurs in ten to twelve days. The course is always slow, and is not materially faster in 1 : 1000 than in 1 : 10,000.

The undeveloped ova show the first effects between eight and thirty-six hours, death occurring in between sixteen to forty-eight hours.

Dose. — As has been pointed out, the 1 : 100,000 solution has almost no effect. The difference between 1 : 10,000 and 1 : 1000 is insignificant in the developed embryo, but decisive in the undeveloped.

The distilled-water solution is perhaps somewhat more toxic than the sea water.

Replacement in pure sea water after ten days' sojourn in the veratrin does not cause recovery, the further course being as in the veratrin solution.

VI. Temporary Suspension of the Development of the Ova by Potassium Cyanid.

On exposing the recently fertilized ova of Fundulus heteroclitus to 1 : 1000 potassium cyanid solution (in sea water), a prompt arrest of development follows, and no sign of life is perceptible. The cells, however, do not disintegrate for two or three days; and if they are transferred to pure sea water, or more dilute potassium cyanid solution, at any time during this period (*i. e.*, up to the second day after apparently complete death), development is resumed after a latent period of one or two days, the embryos hatching normally, but about two to four days after the control specimens.

The following protocol illustrated an experiment of this kind:

Experiment 70. — About a hundred ova are fertilized in sea water. In two and three quarters hours, when they have reached the four-cell stage, they are transferred to 1 : 1000 potassium cyanide, in sea water, in a covered finger-bowl.[1]

Retardation of development in potassium cyanid. —

Time of immersion in potassium cyanid solution.	Development in pure sea water.	Development in potassium cyanid solution.
0.	4 cells.	4 cells.
2¾ hours.	16 cells.	16 cells.
1 day.[2]	Flat disc.	Disc not as flat. Complete arrest of development.

[1] The solution is renewed daily, since another experiment (44) had shown that the solution weakens by standing, and that the ova may resume development after four days in the solution itself. Fresh ova, placed in this weakened solution and left therein, showed a slightly retarded development at first, but continued to develop, and one of the three ova hatched, two days after the control. The others died five days before the time of hatching.

[2] The first appreciable retardation of development was observed in Experiment 44 to occur in between four and one-half and seven and one-half hours.

Time of immersion in potassium cyanid solution.	Development in pure sea water.	Development in potassium cyanid solution.
2 days.	Acardiac embryos.	Disc.
3 days.	Circulation established.	Disc.
4 days.	....	Some are liquefied, others still in disc stage.

Recovery in sea water. — The ova were removed from the potassium cyanid solution at the stated intervals, rinsed in sea water, and then placed in sea water. Three to five ova were used for each test.

Time of immersion in potassium cyanid solution.	Development after transfer to pure sea water.
Momentary .	As the normal control specimens (*i. e.*, those which had not been placed in the cyanid solution).
1 hour . .	Very slightly retarded for one day; then like normal control.
2 hours .	Slightly retarded for one day, then like normal.
21 hours .	Slightly retarded for two days, then like normal. Up to this time hatching occurs at the same time as with the normal.
2 days . .	No apparent development for a day; then development is resumed, keeping about two days behind the control; a second specimen had not hatched, but was alive, nineteen days after the control. Two others died as ova, but completely developed.
3 days . .	No apparent development for two days. Development then proceeds rapidly. One hatches perfectly two days after the control. The other develops, and remains alive but unhatched nineteen days after the control.
4 days . .	No recovery. Liquefaction proceeds.

In no case could recovery be obtained after the protoplasm of the ovum had liquefied. Intactness of the structure of the protoplasm is essential.

Ova, two days old, which had developed into acardiac embryos (Experiment XXI.) appeared dead after nineteen hours' sojourn in the potassium cyanid solution; but when replaced in sea water, after three days' sojourn in potassium cyanid, development resumed within one day, and hatching occurred at the same time as the controls.

Ova, four days old (heart and vessels present), showed recovery after two days' sojourn, but not after three days':

Experiment 72. —

Time of immersion	Changes in potassium cyanid solution
½ hour	No change.
1¼ and 3 hours . .	Heart slowed to 40, and weak.
7 hours . . .	No heart beat.
22 and 46 hours . .	Some are liquefied, but others retain normal structure.
3 days . . .	All are liquefied.

Recovery in sea water. —

Time of immersion in potassium cyanid solution.	Results of transfer to pure sea water.
Momentary .	Development as the normal control specimens.
½ hour . .	Development as per the control, but hatching delayed by four and seventeen days.
1¼ hours . (Heart slowed to 40.)	The heart has partially recovered in five hours after the transfer; rate 56, weak, regurgitation. It is normal on the following day. One hatches five days after the control, two die unhatched fourteen days after the control hatches.
3 hours .	In one hour the heart has recovered somewhat, the sinus and auricle beating at the rate of 64, the ventricle at 32; weak; regurgitation. In three hours the heart is still weak, but is normal on the following day Hatching is delayed by one and four days. Two ova die four and ten days after the hatching of the control specimens.
7 hours . (Heart stopped.)	The heart has returned to normal in one day. The ova developed normally, but none hatched. Death occurred eighteen days after the hatching of the control specimens. The shell was considerably swollen.
22 hours .	In one day two of the ova have liquefied, but one has recovered and hatches three days after the control. The embryo is deformed.
23 hours .	Recovered in one day.
46 hours .	Recovered in one day. One embryo breaks the shell one day after the control, but dies before hatching is completed. The other two embryos die in the same manner eight days after the hatching of the control specimens.
3 days . .	No recovery.

In this experiment the short immersion in the cyanid shows a tendency to interfere with the hatching but not with the development. The interference is not at all proportional to the duration of the immersion.

The recovery of the heart after the standstill of forty hours is remarkable.

Only one experiment was tried with older ova: in Experiment 97, the ova were transferred to the potassium cyanid solution eight days after fertilization. At the end of twenty-three hours they were apparently dead, and no recovery occurred on transferring them to sea water at this time. It would seem, therefore, that the older ova are not capable of recovery.

Hatched embryos and adult fishes never showed any recovery in sea water even after only five hours' exposure to the just fatal concentration of potassium cyanid. Recovery occurred only if the transfer was made before death.

Discussion.

The results of these experiments can be most readily explained on the assumption that the cyanid retards (fermentative?) metabolic processes which result in the disintegration of the protoplasm, as well as those which find their expression in the ordinary phenomena of life, such as cell division and growth, and the muscular activity of the heart. In other words, it appears capable of inducing a prolonged period of suspended animation, when the ordinary phenomena of life are absent, but during which the capability of resuming life persists. (It would be very interesting to determine the gaseous metabolism during this suspended animation.)

This assumption recalls the theory of J. Loeb, which he attempted to confirm by a series of experiments on sea-urchin eggs.[1] It seemed, from these results, that viability of the unfertilized ova could be prolonged for several days by $\frac{n}{1000}$ (0.0647 gm. per 1000 c.c.) potassium cyanid solution. Gorham and Tower[2] threw doubt on this interpretation of the results by showing that the ova remained alive for a still longer period in sterile, pure sea water, and that the cyanid acted as a potent antiseptic.

Gorham and Tower's objections do not apply to my experiments; for all other poisons which killed ova promptly, such as digitalin and veratrin, did not show any recovery. It is not conceivable that in these cases bacteria had entered through the very inpenetrable and resistant egg-shell, in the course of a few hours, in sufficient quantity

[1] J. Loeb and W. H. Lewis: This journal, 1902, vi, p. 305.

[2] F. P. Gorham and R. W. Tower: This journal, 1902, viii, p. 175.

to produce disintegration. This assumption would be necessary if the prolonged suspension of life by the cyanid were to be explained by its antiseptic action.

The fact that this suspension of life occurs only in the younger ova may be explained by the greater delicacy of the more developed structures and the consequent greater tendency to succumb to the disintegrative changes, or by the greater susceptibility to the fatal action of the cyanid.

Conclusions.

Potassium cyanid solutions produce in the younger ova a condition in which the development and the heart action are at a standstill, but during which the structure of the protoplasm and capability of resuming life is preserved. This condition lasts for several days. It was not observed in older ova or in hatched fish. It is presumably explainable by an inhibition of the autolytic metabolic processes which accompany death.

COMPARATIVE PHYSIOLOGY OF THE INVERTEBRATE HEART.—V. THE HEART RHYTHM UNDER NORMAL AND EXPERIMENTAL CONDITIONS.

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THE work on the physiology of the heart nerves in the invertebrates led to the conclusion that the heart of at least most of the invertebrates is supplied with augmentor or inhibitory nerves, in most cases, and probably in all, with both kinds of nerves, just like the vertebrate heart.[1] The demonstration, physiologically, of the presence of cardio-regulative nerves is not attended with any serious difficulties in most of the invertebrates. Nevertheless, the earlier negative results by workers in this field (Foster, Foster and Dew-Smith, Darwin) have almost obtained the force of dogma in the literature on the heart. Thus Beyne[2] in two recent papers takes the supposed absence of all nervous tissue in the snail's heart as a demonstrated fact, without even referring to the papers of Ransom,[3] Pompilian,[4] and myself. It is superfluous to state that conclusions based on the supposed absence of nervous tissue in the snail's heart are erroneous. Ransom demonstrated the presence of nerve fibres in the snail's heart both by histological and physiological methods. Dogiel and Pompilian have described nerve cells in the heart; and my own observations go to confirm and extend Ransom's results.

In this connection I also desire to call attention to Straub's negative results as regards the presence of cardio-regulative nerves in the marine gasteropod Aplysia.[5] Cardio-augmentor nerves have been

[1] CARLSON: Biological bulletin, 1905, viii, p. 123; This journal, 1904, xii, p. 55; 1905, xiii, p. 396; xiv, p. 16; 1906, xv, p. 127.

[2] BEYNE: Journal de physiologie et de pathologie générale, 1905, lxx, p. 949.

[3] RANSOM: Journal of physiology, 1884, v, p. 261.

[4] POMPILIAN: Comptes rendus de la société de biologie société biologique, 1900, lii, p. 185.

[5] STRAUB: Archiv für die gesammte Physiologie, 1904, ciii, p. 429.

described in Aplysia by Schoenlein,[1] and by Bottazzi and Enriques,[2] but Straub remarks that "the results of these investigators are not conclusive because they worked on empty hearts." Does Straub mean to say that the presence of extrinsic heart nerves in an animal is not demonstrated when it has been shown, sources of error being excluded, that stimulation of certain nerves augments or inhibits the empty heart? Or does Straub mean to imply that these nerves find their way into the heart when the heart becomes empty, or that they become functional only in the empty heart? I have not worked on the species of Aplysia found at Naples, but in the case of the representative of the same genus on the Pacific Coast (Southern California) the presence of cardio-augmentor nerves is easily demonstrated by physiological methods, just as Schoenlein and Bottazzi and Enriques have done in the Naples form. It seems highly improbable that heart nerves are present in the one species of a genus and not in the other.

It now remains to summarize the observations on the properties of the heart tissues as revealed by direct stimulation. We shall see that these results lead to a general conclusion similar to that yielded by the work on the heart nerves, namely, that the invertebrate heart exhibits in varying degrees the same reactions and peculiar properties as the vertebrate heart, properties by which the vertebrate heart tissues differ more or less from the skeletal muscle and the smooth muscle. We found that there is no fundamental difference between the vertebrate and the invertebrate heart so far as regards the cardio-regulative nerves. Similarly, the difference between the vertebrate and the invertebrate heart as regards the physiological properties of the heart tissues is only one of degree, not a difference in kind.

I. The Heart Preparation.

In studying the reactions of the heart to direct stimulation the graphic method was employed in all animals whose ventricles or auricles are of sufficient size and strength to make it applicable. The systemic ventricle and the gill ventricles of Octopus, the systemic ventricle of Loligo, the ventricles of the lamellibranchs and most of the gasteropods all permit the ordinary graphic method by suspension. There is a great difference between the size and strength of

[1] Schoenlein: Zeitschrift für Biologie, 1894, xviii, p. 187.

[2] Bottazzi and Enriques: Archives italiennes de biologie, 1901, xxxiv, p. 111.

the systemic heart of a thirty-pound Octopus and the ventricle of Bulla or the auricle of Helix, but by making the power arm of the exceedingly light lever longer than the weight arm even these weaker hearts can be adapted to the graphic method.

Graphic records by means of an upright placed on the heart *in situ* is on the whole impracticable both in the molluscs and the arthropods. The ventricles of the lamellibranchs, the chitons, the diatocardic prosobranchs, the tectibranchs, and the nudibranchs are not compact enough for that purpose. As soon as the heart is emptied of blood the ventricular walls collapse. The ventricle of Natica, Ariolimax, Octopus, the systemic ventricle of Loligo, and the heart of Palinurus or Cancer are organs of sufficient compactness to allow this procedure; but in these, as well as in the other groups, the cardiac apparatus is so closely connected with adjacent contractile tissue or organs that when stimulating the heart *in situ* one cannot prevent the escape of the electrical current to these organs and their subsequent contraction, which interferes with the movements of the heart and renders interpretation of the tracings difficult. This limits us to the method of suspending the excised heart, either empty or filled with blood or saline solution by means of a cannula in the aorta. The ventricles of the lamellibranchs and the diatocardic prosobranchs were usually suspended by means of silk ligatures secured at either auriculo-ventricular junction. For some purposes the ligatures were secured at one of the auricular junctions and at the aortic end around the gut; but this gives less satisfactory results, probably because the tension is less evenly distributed throughout the organ. The penetration of the ventricle by the hind gut in the lamellibranchs and some of the lower gasteropods is a hindrance in the experiments. The gut is so closely invested by the ventricle that the two cannot be separated without much injury to the ventricle. The gut is usually filled with fæcal matter, and its mere weight interferes with the movements of the suspended ventricle.

The ventricle of the chiton is in communication with the auricles by two openings at either side, and can therefore not be suspended by ligatures at auricular junctions. The best results were obtained with the ventricle suspended by means of a ligature around the aortic sinus and a hook in the connective tissue to which the posterior end of the ventricle is attached.

The ventricles of all the monotocardic gasteropods were suspended by means of ligatures around the aorta or aortic sinus and at the

auriculo-ventricular junction. But if this is done with the ventricle of Natica, the tension is unevenly distributed in the organ and only a part of the force of contraction is communicated to the lever, as the aortic and the auriculo-ventricular openings are not at opposite ends of the ventricle, but approach each other so that the distance between them on one side is only one-third that on the other side. A ligature at the auricular junction and a platinum hook in the belly of the ventricle gave the best results.

The full contraction force of the systemic ventricle of Loligo is best attained by suspending the ventricle by means of ligatures around the anterior and the posterior aortæ. But records were also taken from the ventricle suspended by ligatures at the auricular junctions as well as by ligatures at one of the auricular and one of the aortic ends. The systemic ventricle of Octopus was suspended by means of ligatures either at both auriculo-ventricular junctions or at one of the auricular ends and at the main posterior aorta. The gill ventricles of Octopus are suspended to best advantage by a ligature around the afferent branchial veins and a platinum hook in the connective tissue attached to the posterior end of the ventricle, as the opening to the renal veins is not at the posterior end of the ventricle but lateral, so that if the ventricle is suspended by means of a second ligature at the point of union of the kidney and the ventricle, only a part of the contraction is indicated by the lever.

The hearts of the decapod crustaceans were suspended by means of ligatures around the anterior and posterior aortæ or the supporting ligaments at opposite sides, both methods giving equally good results.

The gill ventricles of Loligo, the hearts of the tunicates, and the auricles of most of the molluscs are too delicate for the graphic method. In the molluscs the attention was chiefly directed to the ventricle, because a few experiments on the auricle sufficed to show that the properties of the auricular tissues are in all essentials the same as those of the ventricular. When these small and feeble organs were studied, an indirect method of graphic registration was made use of. I found this particularly useful in studying the rhythm and the reactions of the tunicate heart. But it is plain that this aid to the direct observation is of no service in problems involving the magnitude of contraction, — for example, the refractory period and the response of the heart to stimuli of varying strengths. I found it convenient and quite accurate to measure the strength of contraction in the tunicate heart by aid of the microscope and an eye-piece micrometer.

II. The Normal Heart Rhythm. The Rhythm of the Exposed Heart when left in Situ.

In but very few of all the animals worked on can the heart be observed in the intact specimens, and the rhythm and contraction time of the cardiac apparatus in any way recorded under normal physiological conditions. The required dissections involve changes in intracardiac and extracardiac pressure, and very often mechanical stimulation of the heart itself, so that the exposed but otherwise intact heart works under quite abnormal conditions. The heart of the lamellibranchs was exposed by removing one of the shells and slitting open the dorsal pericardium. In Mya this can be done without any injury to the heart, because the shell is brittle and easily removed. In Mytilus, Cardium, and Hennites the shells had to be pried apart sufficiently to introduce a small scalpel to sever the shell muscles and the adductors of the foot on one side. This is rarely accomplished without injury to the pericardium and the auricle of that side, especially in Mytilus, in which the posterior adductors of the foot and the byssus lie close to the side of the pericardial cavity. The ventricle of Mytilus is very sensitive to mechanical stimulation, the pressure or pulling in the removal of the shell frequently producing a continuous maximal contraction of the ventricle lasting for several minutes. When the shell is removed and the heart exposed without producing this prolonged systole, the rhythm is generally very regular, continuing with gradually decreasing rate of pulsation for one to two hours if the heart is left undisturbed and protected from evaporation. The rate of the beats of the exposed heart varies from 10 to 15 per minute. This is, in all probability, less than the normal, because both auricles and ventricle are empty and collapsed, and we shall see that within limits increase in the intracardiac pressure increases the rate of the beats. When the co-ordination between the auricle and the ventricle is normal, there is a perceptible interval between the auricular and the beginning of the ventricular beats. But frequently the auricles and the ventricle beat with independent rhythm, the auricles usually faster than the ventricle. The ventricles of the other lamellibranchs studied were never observed to go into the prolonged contraction on gentle mechanical stimulation, so characteristic in Mytilus. The rhythm of the exposed and empty ventricle of Mya appears, on the whole, much slower (5 to 10 per minute), the rhythm of the ventricle of Cardium somewhat faster than in Mytilus.

Removal of the foot and the ventral wall of the pericardium in the chitons leaves the heart empty and collapsed, but otherwise intact. The heart is nevertheless under considerable tension, owing to the attachment of the base of the auricles to the rigid framework of the dorsum. That this tension is of importance to the rhythm is shown by the fact that when the auricles in Cryptochiton are severed at their base, both auricles and ventricle cease beating. In Ischnochiton this severance of the auricles does not stop the ventricular rhythm entirely, but the beats become less rapid and regular. The cessation of the auricular rhythm may be ascribed to the injury of the auricles in the operation, but that does not account for the failure of the ventricular rhythm. The ventricle of Cryptochiton usually continues to beat for an hour or more after being thus exposed ; but the rhythm is exceedingly slow and irregular, the rate of the beats varying from 5 to 7 per minute. Perfect co-ordination between the auricles and the ventricle rarely obtains in the exposed heart of this animal, nor do the auricles beat simultaneously. The lack of co-ordination in the exposed and empty heart of Cryptochiton is greater than in any other mollusc worked on. The behavior of the exposed and empty heart of Ischnochiton is very different. The heart of this animal continues to beat with perfect co-ordination and regularity of rhythm for two to three hours after being exposed, the rate of the beats soon after the dissection varying from 15 to 25 per minute.

Exposing the cardiac apparatus in Haliotis involves only the slight injury of separating the mantle and the stalk of the foot from the shell, as the heart can be observed through the dorsal wall of the pericardial cavity. The removal of the shell causes very little bleeding, and consequently but slight change in the blood pressure. The animal minus the shell moves about in the aquarium or on the table for some little time, thus giving an opportunity of studying the action of the heart under nearly normal conditions. The rhythm is variable, violent contractions of the body sometimes augmenting and at other times retarding it. But there always appeared to be perfect co-ordination between the auricles and the ventricle. Exposing the heart by removing the dorsal pericardium accelerates the rhythm for a brief period; then irregularities in the rhythm set in, the auricles beating faster than the ventricle; and finally the ventricle ceases to beat entirely, while the auricles keep up an irregular rhythm for a much longer time. The removal of the shell and exposing the heart in

Lucapina involve greater injuries and loss of blood. The dorsal pericardium is opaque, making it necessary to remove it in order to observe the heart action. Under these conditions the heart rhythm is more regular than in the abalone, but the rate of the beats is somewhat less, or only 12 to 20 per minute. Exposing the heart in Natica or Sycotypus involves extensive injuries to the soft parts of the animal in removing the tough and convoluted shell, and after the shell is removed the permanent contraction of the body is so extreme that all the blood is forced out of the contracted parts. The exposed heart is therefore empty, but the ventricular walls are so compact that they do not collapse. In most of the Natica preparations the heart did not beat at all after being exposed, but in few preparations a feeble and slow (5 to 7 per minute) rhythm was maintained for a short time after the dissection. If protected from evaporation, the heart remains excitable for two to three days.

In Pleurobranchæa the heart rhythm can be observed in the intact and undisturbed specimen in the aquarium by the displacement of the dorsum above the heart. The pulse rate in this animal is relatively low, or only 20 to 30 per minute. As low a rate as 6 to 10 per minute was observed in specimens that were in poor condition. Contraction of the body augments the rhythm. The heart of Bulla can also be observed in the practically intact animal, because the shell is so brittle that its removal involves but slight injury and loss of blood, and after removal of the shell the animal does not, like Natica, remain in extreme contraction. The heart is situated dorsally, and the pericardium is transparent. When the shell has been removed, the heart rhythm is usually quite irregular even to the extent of lack of coordination between the auricle and the ventricle. In the specimens that showed the most constant rhythm the rate of the beats varied from 10 to 18 per minute. The dorsum and the dorsal wall of the pericardium cannot be removed and the heart exposed without producing great variations in the blood pressure and in the pressure on the heart from the violent contractions of the animal when handled or injured. This is particularly true of Aplysia. When being extensively injured, the body musculature of this animal goes into an extreme and prolonged contraction. It is therefore impracticable to endeavor to expose the heart while at the same time retaining the circulation intact. In Aplysia and Pleurobranchæa I found it most convenient to remove the dorsum with the pericardial sac and part of the kidney and the base of the gill from the rest of the body, fix the

dorsum to a board by pins, and expose the heart by cutting away the ventral wall of the pericardial cavity. The behavior of the Aplysia heart in this condition is variable; some preparations exhibit a slow (8 to 15 per minute) but regular rhythm for fifteen to twenty minutes, each auricular contraction being followed by that of the ventricle; but in many cases the rhythm is irregular from the beginning or becomes so within a few minutes after the dissection.

In the nudibranch Triopha carpenteri the heart can be observed in the intact animal through the transparent mantle and pericardium. When the specimens are left undisturbed in a dish of sea water, the rhythm of the heart is very regular, the rate varying from 30 to 40 per minute in different specimens. Locomotion does not affect the pulse rate perceptibly. The ventricular beat is characterized by a very slow systole and a rapid diastole, the former taking more than twice the time of the latter. The heart continues to beat when the animal is pinned to a board and the mantle slit open, despite the extreme contraction of the body and consequent change of pressure in and on the heart; but touching the pericardium or the heart directly with the dissecting instruments sends the ventricle into a prolonged maximal contraction similar to that of the ventricle of Mytilus on mechanical stimulation. The opening of the body cavity leaves the heart empty of blood, and in this condition the heart maintains a slow and irregular rhythm for one to two hours, the auricle beating faster than the ventricle, the rhythm of the latter rarely exceeding 10 beats per minute. In Triopha maculata, Eolus sp., Archidoris, and Montereina the heart cannot be observed in the intact animal because of the pigment in the mantle. The exposed and empty heart of Montereina continues to beat for a few minutes with a perfectly regular rhythm, the rate varying from 20 to 30 per minute; but the co-ordination between the auricle and the ventricle soon ceases, the auricle beating faster than the ventricle. Part of the irregularity in the ventricular rhythm is caused by the lack of co-ordination between the auricular and the ventricular beats, for when the auricle is severed from the ventricle the latter continues to beat with a fairly regular rhythm for more than an hour, the rate of the beats varying from 8 to 16 per minute. When first exposed, the heart of Montereina is more sensitive to mechanical stimulation than the hearts of the other nudibranchs, the slightest touch sending the ventricle into extreme contraction lasting for five to ten minutes.

The excitability soon decreases or changes, so that a gentle mechanical stimulation produces only a single normal beat.

In Ariolimax the heart beat can be observed by the displacement of the mantle when the animal is not too much contracted. The cardiac rhythm in this slug is more regular than that of any other gasteropod under the conditions for observation, the rate scarcely varying from 35 to 40 per minute in healthy individuals. When the animal is pinned to the board and the dorsum or the foot slit open, the blood escapes so that the exposed heart is empty of blood. The auricle is collapsed, but the ventricle only slightly so, as its muscular walls are relatively compact. In this condition the heart continues to beat for several hours with but slightly lower rate than in the intact animal. The co-ordination between the auricular and the ventricular beats soon ceases; but after severing the heart at the auriculo-ventricular junction, each organ continues to beat with an independent but perfectly regular rhythm, the auricle usually ceasing to beat sooner than the ventricle. That the heart of this slug is not very sensitive to external pressure is shown by the fact that the rhythm is hardly influenced by the contraction of the kidney which invests the heart, and, when exposed, the ventricle can be pulled and moved about considerably without affecting the beats, provided it is handled by the aorta or the auricle. Touching the ventricle directly produces an extra beat, and if the mechanical stimulation is very strong, an acceleration of the rhythm bordering on incomplete tetanus for a few seconds. The heart beat does not produce such distinct movements of the mantle in Limax as in the giant slug, but the rhythm of the exposed heart is faster in Limax, the ventricle is more sensitive to mechanical stimulation and dies sooner, the rhythm being rarely maintained for more than an hour after dissection. The behavior of the exposed heart of Helix has been described by Foster and Dew-Smith and by Ransom. The required dissections interfere with the rhythm to a greater extent than in the slugs, as the rate of the beats rarely exceeds 10 to 15 per minute, while under normal conditions the rate is probably not less than 40 to 50 per minute.

The behavior of the cardiac apparatus of the cephalopods when the mantle has been slit open so that the action of the heart can be observed has been described in connection with the physiology of the cardiac nerves. The operation, which can but slightly influence the blood pressure by the loss of blood or by prolonged contraction of any part of the body, completely destroys the co-ordination of the

heart in Loligo, while it hardly affects the activity of the heart of Octopus. But the co-ordination between the different parts of the squid heart is abolished and the beats of the systemic ventricle rendered very irregular, the gill ventricles maintain a regular rhythm for twenty to forty minutes, both when left undisturbed and when separated from the renal veins and the gills. When the mantle cavity is first opened, the gill ventricles of Loligo pulsate with a rate of 70 to 80 per minute. Bottazzi and Enriques[1] give the normal pulse rate in Octopus as 35 per minute, which is considerably higher than in the specimens observed by me. The cardiac rhythm in Loligo is in all probability at least twice as rapid as that of Octopus, or more than 60 per minute. The gill ventricles of Octopus, like those of Loligo, maintain their rhythm for a longer time than the systemic ventricle. I have observed specimens, killed by a blow on the head, in which the gill ventricles continued rhythmical pulsations for two hours after the systemic ventricle ceased beating, the cardiac apparatus being left intact and *in situ*.

When a small puncture is made in the carapace dorsal to the heart in the crabs so that the beat of the heart can be observed by the movements of the dorsal pericardium, the heart rhythm is seen to be very regular. In Cancer the rate of the beats varies from 90 to 120 per minute. The small puncture involves but slight injury and loss of blood, so that the heart is working under almost normal conditions except for the stimulation by the injury. If now the ambulatory appendages are cut off or an opening made into the visceral cavity, the pulse rate falls immediately to from 15 to 30 per minute, and this rhythm is maintained quite regular in the majority of the preparations for thirty to sixty minutes. The extensive injuries leave the heart devoid of blood, which is evidently the cause of the great decrease in the pulse rate. In Palinurus there is less difference in the pulse between the intact heart and the heart that is emptied of blood. The heart of this animal continues to beat for more than two hours after it has been emptied of blood if left intact in the body of the animal.

The heart of Clavellina is too small to be isolated for experimentation. In young specimens the test is transparent, so that the heart action can be studied in the intact animal by aid of a hand lens or the microscope. When older specimens were used, the whole animal was

[1] Bottazzi and Enriques: Archives italiennes de biologie, 1902, xxxiv, p. 111.

usually removed from the test or else the test cut away at the visceral end. The smaller specimens with transparent tests offer the best opportunity for studying the heart action under normal conditions. The periods of reversal of the direction of the pulse wave are separated by pauses. Towards the end of a period there is a distinct slowing of the pulse rate; then follows the diastolic pause for two to four seconds, and the beats commence with renewed rapidity from the opposite end of the heart. The pulse rate decreases rapidly if the animal is kept in sea water not sufficiently aerated. In fresh specimens the rate varies from 30 to 50 per minute. The heart of Ciona can be studied in the intact but greatly contracted animal after removing the test, but for the purposes of these experiments the pericardial sac together with the heart was removed from the animal and placed in a dish of sea water. One side of the heart is so closely attached to the pericardium that it is not possible to sever the connections without so extensive injury to the heart that rhythmical contractions cease. The pericardium was usually slit open and the heart stretched out at its full length. In this condition the heart continues to beat rhythmically for hours. Some peculiar irregularities in the rhythm appeared occasionally. The beats would start at the middle of the heart instead of at either end, or contractions would start simultaneously at both ends of the heart tube. The decrease in the pulse rate towards the end of a series is less evident in Ciona than in Clavellina, and the diastolic pause between the reversed series is frequently wanting.

III. The Rhythm of the Suspended Heart when Empty and when Filled.

The behavior of the empty heart. The effects of mechanical tension. —The ventricle from a healthy specimen of Mytilus continues to beat with perfectly regular rhythm for from four to six hours after being suspended. It frequently happens that the necessary manipulations in preparing and suspending the ventricle produce a great increase in the tonus, on which are superadded a rapid series of diminutive beats, or they may produce a prolonged maximal contraction lasting for several minutes; but if the ventricle is left undisturbed, the tonus returns gradually to its normal condition, and with the beginning of tonus relaxation the fundamental rhythmical beats reappear, at first very rapid, the rate decreasing with the decreasing

tonus until the original tonus condition of the ventricle is reached, when there is no further variation in the rhythm, except a very gradual decrease in the rate and strength of the beats in case the experiment is continued for some hours. The suspended ventricles of Mya, Cardium, and Hennites show less regularity in the rhythm, and the rhythm is not maintained for as long a time as in the Mytilus ventricle.

The ventricle of Ischnochiton beats with regular rhythm for thirty to sixty minutes after being suspended, but when subjected to electrical stimulation irregularities in the rhythm and final cessation of the beats appear much sooner. The suspended ventricle of Cryptochiton, on the other hand, never exhibited any rhythmic activity. More than 30 specimens were tried at different times of the year, but all with the same result.

The suspended ventricles from healthy specimens of Haliotis and Lucapina usually maintain a regular rhythm for fifteen to thirty minutes and irregular contractions for a longer time. But not infrequently variations in the magnitude of contraction and in the length of the diastolic pause appear immediately after the ventricle has been suspended. The suspended ventricle of Natica remains quiescent, the absence of automatic beats being probably due to unavoidable injuries to the heart in taking the animal out of the shell.

The ventricles from healthy specimens of Aplysia, Bulla, and Pleurobranchæa will in the majority of cases beat with perfectly regular rhythm for twenty to thirty minutes after being suspended before irregularities in the rhythm appear. The rate of pulsation in the suspended ventricles of Bulla and Pleurobranchæa is rarely greater, but mostly somewhat less than that of the heart in the intact animal. But ventricle preparations from these molluscs were also met with in which the rhythm was irregular from the beginning. This was more often the case with Pleurobranchæa than with Bulla or Aplysia, probably because all the Pleurobranchæa material at my disposal was brought in by fishermen and most of the specimens probably sustained some injuries in the capture and transfer.

Straub[1] states that by far the greater number of the Aplysia ventricles when suspended and empty give but a few beats and remain quiescent or else the rhythm is irregular, and only rarely is a preparation obtained which continues beating for any length of time with a regular rhythm. That was not my experience either with Aplysia

[1] STRAUB: Archiv für die gesammte Physiologie, 1902, lxxxvi, p. 504.

or the other tectibranchs worked on. To be sure, in the work on Aplysia, comprising over 75 specimens, I frequently worked with ventricles that when suspended gave no beats at all, or at best slow and irregular beats, but when specimens in good condition were selected and the ventricles not injured in the dissection, I nearly always obtained preparations that exhibited a regular rhythm for fifteen to twenty minutes after being suspended. I am inclined to ascribe the absence of and the irregularity in the rhythm in some of the preparations to poor condition of the ventricles and to injuries sustained in the dissection, as uniformity in the rhythm was the rule and not the exception in the ventricles from fresh and vigorous specimens. On the other hand, I rarely obtained such ventricle preparations from the animals which I knew were not in the best condition. For example, out of a lot of ten large specimens which were kept without food for eight days in a cage anchored out in the bay, not one ventricle preparation was obtained that gave a regular rhythm after being suspended in the usual way.

Provided the ventricle of the nudibranch Montereina is prepared and suspended without touching it with the instruments or with the ligatures, it will beat with very constant rhythm for thirty to sixty minutes. Irregularities in the rhythm in the form of grouping of the beats were not observed in this animal. Unless the ventricle is interfered with experimentally, it is usually a case of regular rhythm or else no automatic beats at all.

With only ordinary care in making the dissections one never fails in obtaining ventricle or auricle preparations from the slugs and the snail, which pulsate with perfect rhythm when suspended and weighted with a light lever. I have observed suspended and empty ventricles of Ariolimax beat with a perfect rhythm, of course with gradually decreasing rate and strength of the beats, for ten hours. On being suspended the usual pulse rate in the Ariolimax ventricle is twenty to thirty per minute, which is considerably lower than the pulse rate in the intact animal; but ventricles are not infrequently met with that when first suspended beat with the same rate as when they were in the intact animal, or 35 to 40 per minute. I never observed any irregularities in the way of grouping of the beats either in the snail or the slug hearts; the irregularities that did appear in the rhythm consisted in variations in the strength of contraction.

The systemic ventricles of Loligo usually remain quiescent after being suspended; in some preparations a grouped rhythm appears;

more rarely the ventricles beat with a regular rhythm for five to ten minutes. The ventricles remain excitable for more than an hour. There is scarcely any irregularity in the magnitude of contraction, the irregularity comes in the succession of the beats or in the appearance of more or less definite groups. Two or three such groups may appear in the midst of a rhythmical series of beats without apparent cause, and ventricles which were beating rhythmically sometimes changed into these groups after being stimulated with the interrupted current. The systemic ventricle and the gill ventricles of Octopus beat with a very regular rhythm for more than an hour after suspension. Provided the ventricles are uninjured irregularities in the rhythm in the way of grouping of the beats or variation in the strength of contraction rarely occur. But in order to obtain the effects of the electrical stimulation on the gill ventricles it is necessary to remove their muscular and connective tissue envelope; this cannot be done without more or less injury to the ventricles, in consequence of which these frequently exhibit irregularities in the rhythm when suspended after such preparation. The less the injury to the ventricles the more regular the rhythm, and in few preparations I succeeded in isolating the ventricle with so little injury to it than when suspended the rhythm was perfectly uniform. Ransom states that the gill ventricles are much less suited for physiological experimentation than the systemic ventricle, because the pulse rate is lower and the contraction time of the muscle very much greater than in the systemic ventricle. I am confident that if Ransom had made a trial or two with the gill ventricles from an Octopus in good condition he would have come to a different conclusion. I found practically no difference in rhythm and excitability between the systemic and the branchial ventricles of the same specimen. The pulse rate at the beginning of an experiment on the empty and suspended systemic ventricle varies from 8 to 18 per minute, the usual being 10 or 12 per minute, and the same figures apply to the empty and suspended gill ventricles. It is only in the dying condition of the gill ventricles that mechanical stimulation produces only a local contraction, or, when very strong, a "tonus" contraction lasting a minute or more. The freshly excised and uninjured gill ventricle responds to a mechanical stimulus with a normal beat, provided the stimulus is strong enough to produce any effect at all.

The ventricles from healthy specimens of Cancer or Pachygrapsus, suspended and kept moist by the blood plasma, will continue in uni-

form rhythm for a half hour or more, if left undisturbed, and remain excitable for a considerable time after automatic activity has ceased; but on being subjected to electrical stimulation both rhythm and excitability fail much sooner. Hearts from less vigorous individuals or hearts that were injured in the dissection usually exhibited a very irregular rhythm, three or four beats following each other in rapid succession, in some instances so rapidly that they fused, followed by a pause or greatly prolonged diastole. This peculiar rhythm was kept up for five to ten minutes in some preparations. Grouping of the beats and variations in the strength of contraction usually took the place of the regular rhythm some little time before the suspended ventricle ceased to beat, and often after it had been subjected to strong or long-continued electrical stimulation. The suspended heart of Palinurus maintains a uniform rhythm for a longer time than the heart of the crab, and grouping of the beats and other irregularities in the rhythm are less frequent. The pulse rate of the empty and suspended ventricle of Palinurus varies from 30 to 50 per minute, the usual being about 35. The crab ventricles exhibit greater variations in the pulse rate; while the majority of the suspended hearts would beat with a rate of 35 to 50 per minute, preparations were also met with showing as low pulse rate as 15 to 25 per minute. It would thus appear that the heart of the lobster is less sensitive to the disturbances attendant upon preparation and suspension, while both the lobster heart and the crab heart exhibit a lower pulse rate when suspended and weighted with the recording lever than under normal conditions in the intact animal.

We have thus seen that with the exception of the hearts of Cryptochiton and Natica, the empty and suspended auricles and ventricles act for some time after being suspended as under normal conditions so far as the regularity of the rhythm is concerned. And by a comparison of the behavior of the isolated and empty hearts left *in situ* or in the blood plasma with the behavior of the same hearts when suspended and weighted with the recording lever, the further fact comes to light that *suspending and loading the hearts augment the rate and the strength of the beats and tend to render the rhythm uniform.* This is true without exception, but it is more marked in the molluscan than in the crustacean heart. A ventricle of Helix, which *in situ* and collapsed beats with the rate of 10 to 15 per minute, will usually beat with the rate of 35 to 40 per minute when suspended. The efficacy of mechanical tension on the heart walls as a stimulus to rhythmic

activity is most beautifully illustrated in the heart of the pulmonates. The suspended ventricles and auricles of these molluscs that have been subjected to the experimental conditions until greatly fatigued but which still beat with a perfectly regular rhythm at the rate of 15 to 25 per minute, cease to beat and remain in diastole when the tension is removed by supporting the lever, and when the tension from the lever is again allowed to act on the preparations the rhythm reappears. That mechanical tension, within limits, acts as stimulus to rhythmic activity in the invertebrate heart has also been pointed out by Schoenlein for the ventricle of Aplysia.[1] But while it is true both for the molluscan and the crustacean heart that mechanical tension acts as a stimulus to the rhythm, it is also evident from my observations that tension thus applied almost invariably fails to bring the pulse rate up to what it is in the intact animal. This is certainly true of the ventricles of Bulla, Pleurobranchæa, and Ariolimax, in which animals the pulse rate can be determined in the intact specimen.

The effect on the rhythm of filling the suspended heart. — It has already been stated that lowering the intracardiac pressure reduces the pulse rate. Ransom has shown for the systemic ventricle of Octopus, Biedermann[2] for the ventricle of Helix, and Straub for the ventricle of Aplysia, that filling the ventricle with plasma or physiological saline solution augments the rhythm over that of the empty ventricle, and that within limits the greater the pressure of the liquid in the ventricle the greater the strength of the contraction and the rapidity of succession of the beats. Neither of these investigators states whether the pulse rate of the isolated and artificially filled ventricle is the same or greater or less than that of the ventricle under normal conditions. So far, then, there is a close analogy between the effects on the heart of mechanical tension and of filling the heart with plasma or a saline solution: both produce augmentation of the rhythm. It is not within the scope of this paper to consider what part is played by hydrostatic pressure, by chemical action, or by solution and removal of the waste products of tissue metabolism in producing the effects observed on filling the heart. The point I am coming to is this: are the conditions of the isolated but empty and loaded ventricle so different from those of the isolated and filled ventricle that, *sensu stricto*, the empty ventricle is to be considered in

[1] SCHOENLEIN: Zeitschrift für Biologie, 1894, xviii, p. 187.

[2] BIEDERMANN: Sitzungsberichte der Wiener Akademie, 1884, lxxxix, 3, p. 19.

abnormal, the filled ventricle in a normal, condition? This is the position taken by Straub. Whether the mere filling of the cavity of the ventricle alters the properties of the automatic tissue must be determined by experiment, and it will be referred to in connection with the reaction of the heart to electrical stimulation. But in this connection it may properly be considered with what propriety the one or the other condition of the ventricle is called abnormal, as it involves the principle of experimental method. If Straub's position is the correct one, the properties of the cardiac tissues must be studied in the filled heart; and it follows that the results of Foster and of Foster and Dew-Smith on the heart of the snail, and the results of several investigators on the heart of the crustaceans, only go to show the properties of the cardiac tissues under abnormal conditions, because these investigators worked on the empty heart. Straub makes, indeed, that very deduction regarding Schoenlein's results on the ventricle of Aplysia. Schoenlein introduced unnecessary difficulties in his work by narcotizing the animals with pelletierin before isolating the heart, which in some measure justifies Straub's assertion that the ventricle preparations used by Schoenlein were not under normal conditions. The filling of the heart under conditions that make accurate graphic records possible introduces additional difficulties, especially in the case of small and feeble organs like the auricle and the ventricle of the snail, as well as in the case of the ventricles that are traversed by the gut. Nor is the filling easily accomplished in the hearts of the chitons and the crustaceans.

As long as the ventricle or the auricle continues in uniform rhythmical activity the properties of these organs cannot have undergone any essential change; and we have seen that from all the animals worked on, with the exception of Cryptochiton and Natica, preparations can be obtained, which exhibit a uniform rhythm when suspended in the empty condition. In the case of the ventricle of Cryptochiton and Natica, in which tension failed to produce or maintain rhythmic activity, filling the ventricles with blood or sea water proved equally ineffective. A comparation of the rhythm maintained by the suspended empty and the suspended filled ventricle of Aplysia and Ariolimax brings out the following. The suspended and empty ventricle of Aplysia beats with a rate of from 12 to 20 per minute, and rarely continues in regular rhythm for more than an hour under the experimental conditions. The suspended and filled ventricle of the same animal beats with a rate of from 15 to 35 per minute, and

continues in uniform rhythm for four to six hours. The fact of augmentation of the pulse rate by the filling is readily shown in the same ventricle by first suspending it empty and while suspended filling it with plasma by means of a cannula in the aorta. Augmentation of the rhythm invariably follows the filling. And what is more, if the suspended and empty ventricle exhibits irregularities in the rhythm or no rhythmic activity at all, the filling usually changes the irregular to a uniform rhythm and starts a rhythmical series of beats in the quiescent ventricle. Irregularities in the rhythm of the filled ventricle appear less often. The effect of the filling is less marked in the ventricle of the slug. The empty suspended ventricle of this mollusc beats with perfect rhythm for six to ten hours, the rate soon after being suspended varying from 30 to 35 per minute. Filling the ventricle usually increases the rate by 4 or 5 beats per minute, but the rhythm is not maintained much longer in the filled than in the empty heart.

Ransom worked with the filled systemic ventricle of Octopus. He does not give the pulse rate of the filled ventricle, but the published tracings indicate a rate of from 16 to 20 per minute. In my experiments the systemic and the branchial ventricles of Octopus usually showed a pulse rate of from 12 to 20 per minute; but a uniform rhythm was rarely maintained for more than an hour, while according to Ransom the filled systemic ventricle continues in regular rhythm for several hours.

These facts indicate that *tension on the ventricle exerted by a pull at either end is less efficient than the filling of the ventricle in starting or maintaining the rhythm.* But the difference is only one of degree, and in some animals (Ariolimax) it is insignificant. Hence I do not agree to Straub's contention that in one case the heart is under normal, in the other case under abnormal, conditions. In the strict sense of the word the preparation is under abnormal conditions in either case, for under the experimental conditions the pressure of the liquid is constant, while in the normal condition the pressure is intermittent, not to mention other differences made necessary by the experiment.

In recording the difference in the response to electrical stimulation of the empty and the filled ventricle of Aplysia, Straub does not seem to take into account the change in form of the ventricle as a physical conductor caused by the filling. The filling of the suspended and loaded ventricle of Aplysia increases the cross-sectional area of the

preparation as a physical conductor 5 or 6 times. This fact is sufficient to account for some of the reduced excitability of the filled as compared to that of the empty ventricle. Nothing is as yet known of the relative electrical resistance of the ventricular muscle and the blood in Aplysia. Alt and Schmidt[1] found in some vertebrates that the electrical resistance of the cardiac muscle is less than that of the blood. If the relation should change to be the reverse in Aplysia, it is obvious that only a very small fraction of the current enters the tissues in the filled ventricle.

It is thus evident that mechanical tension on the heart walls and hydrostatic pressure in the heart cavities act as stimuli to the heart rhythm in the invertebrates in the same manner as they do in the vertebrates. The mechanism by which the tension and the intracardiac pressure produce the augmentation will depend on whether the automatism of the heart resides in the nervous or in the muscular tissues. In the heart of Limulus I have demonstrated that neither the tension on the heart walls nor the intracardiac pressure stimulate the heart to activity after the automatic ganglion or dorsomedian nerve cord has been extirpated.[2] In the heart of this animal mechanical tension and intracardiac pressure act in the same way as on all other hearts as long as the ganglion and the nerve plexus is intact. In Limulus the *strengthening* of the beat following increased tension or pressure in the heart cavity may be due to direct action on the heart muscle in a way to increase its excitability and contractility; the augmentation of the rate can only be due to a direct stimulating action on the ganglion, or to stimulation of afferent nerves or nerve endings in the heart walls. The latter would make the mechanism a true reflex through the intrinsic heart ganglion or nerve plexus. If, as seems probable to me, the normal rhythm in all hearts, vertebrate and invertebrate, is neurogenic, the mechanism by which mechanical tension and hydrostatic pressure augment the rhythm is almost certainly the same for all hearts, that is, the same as in the Limulus heart. Dogmatic statements on this point serve no purpose. We need further facts. But in this connection I wish to call attention to two recent papers which bear on this question and seem to give some support to the view just stated. Lingle has shown that, contrary to the commonly accepted view, mechanical tension on strips from the apex of the tortoise ventricle does not produce

[1] Alt and Schmidt: Archiv für die gesammte Physiologie, 1893, liii, p. 575.

[2] Carlson: This journal, 1905, xii, p. 487.

or maintain a rhythm.[1] The strip containing in its tissues the normal combination of inorganic (and perhaps organic) constituents of the blood can thus not be made to beat rhythmically by tension alone. On the above interpretation the strip has been removed from the reflex centre, or the part of it in the apical strip is not able to function owing to the injuries of the lesion. The recent observations of Magnus[2] and Sollmann[3] on the rhythm of the excised mammalian heart on perfusion of the coronary vessels with hydrogen, oil, and metallic mercury have shown that the mere mechanical distension of the coronary vessels is able to start and maintain for thirty to forty minutes a rhythm in a quiescent heart. The purely mechanical distension of the walls of the coronary vessels is, of course, an ever-present factor in the normal heart activity, and it may be one of the necessary factors in the complex chain of processes summed up in the phrase "the cause of the heart beat." Sollmann has already pointed out that the stimulating action of the distension of the walls of the coronary vessels may be more readily explained on the neurogenic than on the myogenic theory. On the neurogenic theory afferent fibres or nerve endings in the walls of the coronary vessels are stimulated by the distension of the walls, the impulses passing to the nerve and ganglion plexus in the heart to give rise to afferent impulses to the heart muscle, a mechanism identical with the one I have shown to exist in the Limulus heart. If the mammalian heart rhythm is myogenic, it is difficult to explain why pressure in the ventricular cavities is not just as efficient or even more efficient a stimulus to rhythm than the pressure in the coronary vessels.

[1] LINGLE: This journal, 1905, xiv, p. 449.

[2] MAGNUS: Archiv für experimentelle Pathologie und Pharmakologie, 1902, xlvii, p. 200.

[3] SOLLMANN: This journal, 1906, xv, p. 121.

COMPARATIVE PHYSIOLOGY OF THE INVERTEBRATE HEART.—VI. THE EXCITABILITY OF THE HEART DURING THE DIFFERENT PHASES OF THE HEART BEAT.

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THE reactions of the heart or parts of the heart to direct stimulation are almost universally interpreted as indicating the physiological properties of the heart muscle. Thus some of the properties of the heart muscle are held to be peculiar to the heart, and more or less fundamentally different from the properties of skeletal muscle on the one hand and smooth muscle on the other. This interpretation rests on the assumption that the reactions of the heart or parts of the heart to direct stimulation are not, in part, due to or influenced by the stimulation of the intrinsic nervous tissue in the heart. The physiological properties of skeletal muscle have been determined on curarized preparations. While curare prevents the motor nervous impulses from reaching or affecting the skeletal muscle, the evidence in support of the usual interpretation that the curare produces this effect by paralyzing the motor end plates is not too cogent for Langley to support the very opposite explanation, or that curare blocks the influence of the motor nerves on skeletal muscle by acting directly on the muscle substance rather than on the nerve endings.[1] Langley extends his interpretation to the action of atropin, pilocarpin, etc., arguing that these and related drugs do not paralyze the secretory nerve endings or the nerve endings in smooth muscle, but block the nervous impulses by a direct action on the gland cells and the muscle cells.

It is well known that atropin and some other drugs paralyze the inhibitory heart nerves, but it is a matter of conjecture at what point in the inhibitory nervous path this blocking takes place. In the

[1] LANGLEY: Journal of physiology, 1905, xxxiii, p. 374.

Limulus heart I have demonstrated that atropin, nicotin, and curare block the inhibitory nervous impulses by action on the ganglion.[1] It is also well known that the character of the response of the heart to direct stimulation is altered to some extent by treatments that block the inhibitory nervous impulses.

As regards the augmentor nerves and the intrinsic nervous plexus in the heart, we have no direct evidence that these can be paralyzed by any drug or experimental contrivance that does not at the same time affect the heart muscle. In fact, so far as the evidence goes, it points to the very opposite conclusions, as has been shown for the vertebrate heart by Hering[2] and for the Limulus heart by myself.[3] In Limulus the influence of the intrinsic motor nerve plexus on the heart muscle is not abolished by such drugs as atropin, curare, and nicotin till the excitability of the muscle to direct stimulation is entirely or almost entirely lost. When stimulating the vertebrate or the invertebrate heart directly, we are therefore dealing not only with the effects of direct stimulation of the heart muscle, but also with the effects of simultaneous stimulation of the intrinsic nervous plexus. The question is not simplified by using strips from the ventricular apex, for the entire ventricle contains the nerve plexus or nerve fibres, even if the ganglion cells are less abundant or entirely wanting in the apical third of the ventricle of some animals.[4]

We have scarcely made a beginning in obtaining direct knowledge of the physiology of nerve nets or nerve plexuses, such as we find in many invertebrates and in the heart and the other visceral organs of the vertebrates. Hence we can hardly do more than conjecture as to the part played by the nerve plexus in the response of the heart to direct stimulation. I have shown that the response of the Limulus heart to direct stimulation is altered by the extirpation of the heart ganglion. Bethe's[5] experiments on the nerve net in the bell of the Medusæ, and Magnus'[6] recent work on the nerve plexus in the intestine of mammals are very suggestive in this connection. According to Magnus the wall of the cat's intestine exhibits a refractory state or condition of diminished excitability at the beginning of an auto-

[1] Carlson: This journal, 1905, xiii, p. 238.

[2] Hering: Archiv für die gesammte Physiologie, 1903, xcix, p. 250.

[3] Carlson: Science, 1904, xx, p. 684 (Preliminary communication).

[4] Carlson: Archiv für die gesammte Physiologie, 1905, cix, p. 51.

[5] Bethe: Anatomie und Physiologie des Nervensystems, 1903, pp. 408–456.

[6] Magnus: Archiv für die gesammte Physiologie, 1903, civ, p. 525; 1906, cxi, p. 152.

matic contraction. The automatic or rhythmical contractions appear only in preparations having the plexus of Auerbach intact. The refractory condition does not appear at the beginning of an artificially produced contraction after the plexus of Auerbach has been removed. It would thus seem that the refractory state depends on the plexus of Auerbach, either directly or indirectly through its influence on the muscle. In a more recent paper Schultz[1] states, however, that he has been unable to confirm the above observations of Magnus. But whatever be the factor of the intrinsic nervous tissue in the response of the heart to direct stimulation, the fact should be clearly recognized that no heart preparation so far obtained is composed solely of muscular (and connective) tissues, and that in consequence we have as yet no means of knowing the physiological properties of the heart muscle apart from the nerve plexus. I shall therefore speak of the response of the heart tissues to direct stimulation rather than the response of the heart muscle, leaving to further inquiry what part is played by the two tissues in these reactions.

I. Latent Period and Contraction Time.

The latent period of the heart varies with the condition of the heart and the strength of the stimulus, especially in the hearts of the lamellibranch and the gasteropod molluscs. The decrease in the latent period with the increase in the intensity of the stimulus is less marked in the crustacean and the cephalopod heart. The latent time increases greatly with the increasing fatigue of the heart.

When induction shocks of very great intensity are employed and the heart preparations are in good condition, the latent time in the hearts of the lamellibranchs and the gasteropods does not exceed 0.03 second. The latent period appears to be slightly longer in the lamellibranch and the chiton heart than in the heart of the pulmonates. The ordinary graphic method does not allow very accurate measurements in the small and feeble preparations. I believe photographic registration will show the latent period of the cardiac tissues of these molluscs to be less than 0.02 second. The induced shock of the intensity employed to obtain the shortest possible latent time does not produce a single normal beat, but the beat is accompanied by a prolonged tonus or tetanus contraction. When an induced shock is used of an intensity that does not affect the tonus, the latent

[1] Schultz: Archiv für Physiologie, 1905, Supplement, p. 23.

time appears invariably greater. In the ventricle of Mya it is frequently as large as 0.2 second; but in none of the animals examined did I ever observe a latent period approaching that given by Schoenlein for the ventricle of Aplysia, or 1.5 second.

When allowance is made for the inertia of the apparatus, the tracings show that the latent period of the systemic ventricle of the cephalopods does not exceed 0.02 second, that of the squid ventricle being slightly the shortest. In the crustacean heart the latent time falls considerably below 0.02 second. A more accurate method would probably show that it does not exceed 0.01 second.

Comparative measurements bring out the fact that in the crayfish (Palinurus), the squid, and the slug (Ariolimax), the latent period of the cardiac tissues is the same as that of the body muscles, and I have no doubt that a comparison in other molluscs would bring out the same relation. The cardiac tissues and the body muscle of the molluscs and the crustaceans do not therefore exhibit the great difference in latent time that obtains between the latent period of the cardiac tissues and the skeletal muscle in the vertebrates.

In the ventricle of the lamellibranchs and the prosobranchs the systole and diastole occupy 2 to 3 seconds. In the ventricle of Ischnochiton the contraction time is very nearly the same, but in Cryptochiton it is more than twice as long. In the ventricle of the tectibranchs it approximates 2.5 second, in the nudibranch Montereina and in the giant slug (Ariolimax) it is normally 1.75 second, while in the smaller nudibranchs (Triopha, Eolus), the snail and the garden slug (Limax) the contraction time is about one second. In the ventricles of Octopus the systole and diastole occupy about two seconds, but in the empty and suspended preparations the contraction time is usually longer. The quickest hearts worked with are those of the squid and the crustaceans, the contraction time in these hearts not exceeding 0.25 second. The heart of the lobster is slightly slower in its action than that of the crab.

The myograms from the ventricles of the cephalopods are characterized by the absence of the systolic plateau, which usually appears in the myograms from the ventricles of the lamellibranchs and the lower gasteropods. The ventricular contraction of the nudibranchs and the pulmonates is characterized by a very gradual systole which passes into a more or less distinct systolic plateau, followed by a very quick relaxation, the latter phase occupying less than one-third of the contraction time. No comparisons by means of the graphic method

have been made, but direct observation makes it evident that in the lamellibranchs and the gasteropods the contraction time of the auricle (or auricles) is considerably less than that of the ventricle.

The latent period of the tunicate heart cannot be measured very accurately by direct observation, but it is of greater duration than in the molluscan and crustacean heart. In Clavellina it is probably not less than 0.1 second. The contraction time of the tunicate heart is the time required for the contraction wave to travel from one end of the heart to the other, as under normal conditions the contraction always starts at one end and travels towards the opposite end. But even if we succeeded in measuring the rate of propagation of the contraction wave, that would not constitute a measure of the time occupied by the contraction and relaxation of the muscle, because the heart is ordinarily fully relaxed and frequently started in a second contraction before the previous contraction wave reaches the opposite end of the heart. The contraction and relaxation of the heart at any one point probably occupy not more than 0.25 second when the heart is in good condition. It is far less difficult to measure the rate of propagation of the contraction wave along the heart, though, as far as I know, it has not yet been done. Schultze[1] states that he was unable to do so. By the following device quite accurate measurements can be obtained. When the animal is placed so that both ends of the pulsating heart can be seen plainly at the same time, the beginning of the beat at one end of the heart and the arrival of the contraction wave at the opposite end can be readily made out by aid of a hand lens, or in the case of the Clavellina heart by aid of the microscope. The time interval is most accurately determined by aid of an electromagnetic signal writing on the kymograph, the contact being made at the moment the contraction appears at one end and the circuit broken the moment the contraction wave reaches the other end. At the end of the experiment the heart is isolated and its length measured. If the propagation of the pulse wave involved only a fraction of a second, that is, if the length of the Ciona heart was not so considerable (4 to 4.5 cm.), this method of registering the beginning and the end of the contraction would be of little value, because of the variations in the reaction time of the observer; but as the time interval ranges from one to two seconds in the hearts from the largest specimens of Ciona, these variations constitute only a small percentage of error. In an experimental series only every other or every

[1] SCHULTZE: Jenaische Zeitschrift für Naturwissenschaft, 1901, XXXV, p. 221.

third contraction wave was timed, because in vigorous specimens a new pulse wave usually starts at one end of the heart before the previous contraction has reached the opposite end. *The rate of propagation of the contraction wave in hearts of Ciona beating with a rapid and regular rhythm varies from 2 to 3.5 cm. per second.* The rate is very constant in the same preparation as long as a constant rhythm is maintained, but conditions that decrease the pulse rate decrease at the same time the rate of propagation of the contraction wave, so that in hearts in poor condition but still beating automatically as low a rate of propagation as one-half or 1 cm. per second was frequently observed. But under normal conditions the rate is very nearly 3 cm. per second.

II. The Excitability of the Heart during the Different Phases of the Heart Beat. The Refractory Period.

The first question to be determined in studying the response of the hearts to direct stimulation is the presence or absence of rhythmical variations of the excitability and contractility similar to that exhibited by the cardiac tissues of the vertebrates. According to some authors the vertebrate heart is strictly inexcitable at the beginning of systole; while others use the term "refractory period" in the sense of a condition of greatly reduced excitability only, I shall use the term "refractory period" in the latter sense.

This question has received little attention in the invertebrates. Ransom[1] has published tracings showing a condition of reduced excitability during systole in the systemic ventricle of Octopus. Ransom uses the term "refractory period" loosely, or as meaning both inexcitability and reduced excitability. Schoenlein[2] tacitly assumes the presence of the refractory period for the heart of Aplysia, while Straub[3] maintains that the question must remain undecided forever so far as the heart of Aplysia is concerned, because he finds that the filled ventricle of this mollusc is not affected by single induced shocks. This assumes that the question can be determined only on the filled heart, and that single induction shocks are the only available stimuli for such experiments. Hunt, Bookman, and Tierney[4] found that the

[1] Ransom: Journal of physiology, 1884, v, p. 261.

[2] Schoenlein: Zeitschrift für Biologie, 1894, xviii, p. 187.

[3] Straub: Archiv für die gesammte Physiologie, 1901, lxxxvi, p. 504.

[4] Hunt, Bookman and Tierney: Centralblatt für Physiologie, 1897, xi, p. 274.

heart of the lobster (Homarus) does not exhibit any refractory period under ordinary conditions of temperature. From experiments with the effects of the induced shock on the heart of Salpa, Schultze[1] concludes that the tunicate heart exhibits the systolic refractory period characteristic of the vertebrate heart: "The conductivity for the motor stimulus and the irritability of the muscle fibres is diminished or wholly suspended" (p. 312).

Before stating the results obtained by me, a word may be said of the method of experimentation and choice of stimuli made moot by Straub's contention regarding the Aplysia heart. If the refractory period means a condition of inexcitability of the heart, it must imply inexcitability not only to the single induced shock but to any and all stimuli. The use of thermal and chemical stimuli is attended with almost insurmountable difficulties, but not so mechanical stimuli and the direct electrical current, which when of sufficient intensity excite all excitable tissues. There is one limitation to the use of the direct electrical current as stimulus for testing the condition of excitability of the heart, namely, its electrolytic and polarizing action. It is conceivable that in case the systolic refractory period is of very short duration the disturbance in the heart due to electrolysis and polarization might be of sufficient intensity and sufficient duration to cause an excitation at the return of excitability. Hence, from the failure to find a refractory condition by aid of the direct current, one could not conclude that such a condition is not present. If, on the other hand, the make or the make and break of the direct current which produces an extra contraction when applied to the heart during diastole or the diastolic pause, fails to affect the heart when applied at the beginning or during systole, it seems to me that a condition of inexcitability or reduced excitability is shown quite as conclusively as if single induced shocks had been employed. Furthermore, the failure of a stimulus to produce an extra contraction or a contraction of increased strength does not prove the inexcitability of the heart to that particular kind and strength of stimulus. Such a condition of the heart can be allowed to exist only in case the stimulus produces no effect at all either on the fundamental rhythm or on the tonus of the heart.

In my own experiments the excitability of the heart was tested during the different phases of the automatic as well as the artificially produced beats. The tests were made both with the induced and the

[1] SCHULTZE: Jenaisches Zeitschrift für Naturwissenschaft, 1901, xxxv, p. 221.

constant current, and in the case of the hearts of Aplysia and Ariolimax, both on the empty and the filled ventricle. The tracings obtained in these experiments prove conclusively that *the hearts of all the molluscs, arthropods, and tunicates examined exhibit a condition*

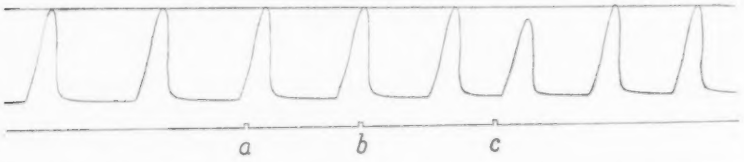

FIGURE 1. — About four-fifths the original size. Tracing from the beating ventricle of Ischnochiton. The break shock sent through the ventricle during systole (*a*) and diastole (*b*) is without effect, but towards the end of the diastolic pause it produces a beat (*c*).

of reduced excitability during systole, the decrease in the excitability being greatest at the very beginning of the systole. But it is a state of diminished excitability only and not a condition of inexcitability, as an intensity of the stimulus can be found sufficient to affect the heart in any phase of the contraction.

Let us first turn our attention to a few typical tracings that show a condition of reduced excitability during systole. The tracing

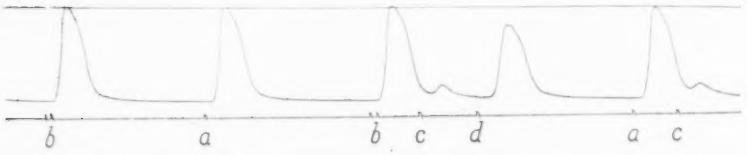

FIGURE 2. — Four-sevenths the original size. Tracing from the empty and quiescent ventricle of Aplysia, stimulated with break induced shocks. The second stimulus effective only towards the end of diastole (*c*) and during the diastolic pause. Showing reduced excitability at the beginning of an artificial beat.

reproduced in Fig. 1 is from the pulsating ventricle of Ischnochiton. The break induced shock sent through the ventricle at the beginning of systole (*a*) as well as towards the end of diastole (*b*) is without effect, but when sent through the ventricle towards the end of the diastolic pause (*c*) an extra contraction is produced. The strength of the shock remains the same. Fig. 2 is from an empty and quiescent ventricle of Aplysia. The single break shock at *a* produces a beat of the same strength as that caused by the two shocks at *b*.

The second shock at *b* passes through the ventricle at the very beginning of systole. The same shock applied to the ventricle towards the end of diastole produces a diminutive beat (*c*), and when the ventricle is fully relaxed a still stronger contraction follows the stimulation (*d*). Fig. 3 is from the filled ventricle of Aplysia. The stimulus is the direct current of a strength that affects the ventricle only on the make. When this current is sent through the ventricle at the beginning or during systole (*a*), it is without effect or in some cases produces a slight diminution in the height of contraction; but when

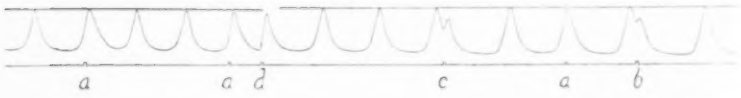

FIGURE 3. — About one-half the original size. Tracing from the filled and beating ventricle of Aplysia, stimulated with the galvanic current. Stimulus ineffective during systole (*a*), but effective during diastole (*b*, *c*) and the diastolic pause (*d*).

sent through the ventricle during diastole, the resulting extra beat is the stronger the more complete the relaxation at the time of stimulation (*b*, *c*, *d*). The tracing in Fig. 4, *A*, is from a resting ventricle of Bulla. The direct current of short duration is used as stimulus. On comparing the beats at *b* with the beats at *a*, it is obvious that the second stimulus at *b* is ineffective. At *c* and *d* the second stimulus becomes effective, the contraction at *d* being greater than at *c*, because the latter follows closer upon the preceding maximal beat. The ventricle of Ariolimax makes a very excellent preparation for the demonstration of the rhythmic variation in excitability. The tracing in Fig. 4, *B*, is from a quiescent ventricle of this pulmonate, stimulated with single induced shocks. By comparing the beats at *a* with the beats at *b* and *c*, it is obvious that the shocks reaching the ventricle during systole produce no visible effects. At *d*, where the fourth shock is applied to the ventricle at the beginning of diastole, the relaxation is slightly delayed, and this delay is more marked at *e*, where the second stimulus is sent through the ventricle in the middle of diastole. The same shock reaching the ventricle at the end of diastole produces a diminutive beat (*f*), and the further on in the diastolic phase the ventricle is stimulated the greater the contraction until a maximal beat is produced when, in this preparation, the stimuli are five seconds apart. The conditions are exactly the same in the pulsating ventricle, as will be seen by referring to Fig. 4 *C*.

The make induced shock is inefficient at the beginning of systole (*a*), and it produces but a slight contraction near the end of diastole (*b*), but towards the end of the diastolic pause a stronger extra beat is produced (*c*).

The condition of reduced excitability during systole is less readily demonstrated in the heart of the squid than in the heart of any other mollusc worked on. In the fresh and vigorous hearts the whole contraction time is but one-fourth of a second, and the processes of

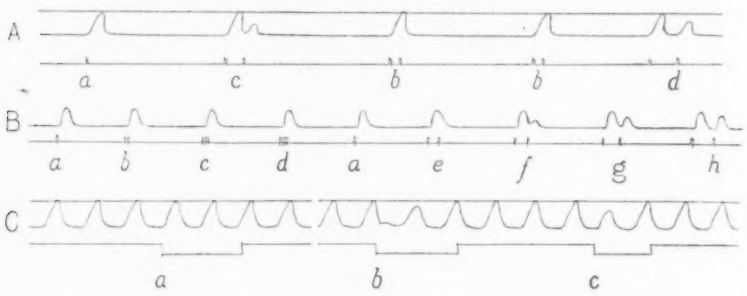

FIGURE 4. — Four-fifths the original size. *A*, Tracing from the quiescent ventricle of Bulla, stimulated with the galvanic current. The stimulus ineffective during systole and diastole of the artificial beat (*b*), but effective at the end of diastole (*c*). *B*, tracing from the quiescent ventricle of Ariolimax. The stimulus (break induced shocks) ineffective during systole (*b, c, d*), but effective towards the end of diastole (*e, f, g*). *C*, tracing from the beating ventricle of Ariolimax, stimulated with the make induced shock. Stimulus ineffective at the beginning of systole (*a*), but effective towards the end of diastole and during the diastolic pause (*d*).

recuperation therefore very rapid. But by using induced shocks of submaximal strength it can be shown, however, that the pulsating ventricle of Loligo does not stand apart from the hearts of other molluscs in this respect. The difference is only one of degree, the state of reduced excitability in the squid heart being of comparatively short duration. In the fatigued and quiescent squid ventricle the reduced excitability during systole is in greater evidence.

The heart of the crab resembles closely that of the squid as regards the appearance of diminished excitability during systole. The systolic decrease in the excitability is very marked in the fatigued and quiescent hearts, but in the fresh and vigorously pulsating hearts the strength of the stimulus must be delicately adjusted and the stimulus reach the heart very near the beginning of systole to prove ineffective. But the duration of the systole is so short that when the circuit

is made or broken just as the change in the form of the heart appears, the stimulus reaches the heart at best towards the end of systole. The less rapid heart of the spiny lobster (Palinupus) exhibits the rhythmic variation in the excitability to a greater degree. A typical tracing from the heart of this decapod is reproduced in Fig. 5. The break induced shock produces no visible effect when sent through the heart during systole (*a*), but at the end of systole the stimulus produces a very slight contraction and a consequent prolongation of the diastolic phase (*b*). The stimuli that reach the heart in the

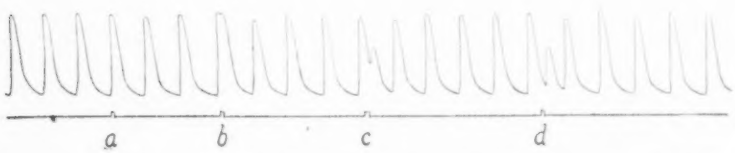

FIGURE 5. — Four-fifths the original size. Tracing from the beating heart of Palinurus, stimulated with break induced shocks. Stimulus ineffective at the beginning of systole (*a*), but effective during diastole (*b*, *c*, *d*), showing reduced excitability during systole.

middle or towards the end of diastole produce correspondingly greater beats (*c*, *d*). It is therefore evident that the crustacean heart falls in line with the molluscan heart. In the hearts of both phyla there obtains a condition of reduced excitability at the beginning of systole. And this is also true for the tunicate heart. My results on the heart of Clavellina and Ciona agree with those of Schultz on Salpa, as regards the existence of a diminished excitability during systole, but contrary to Schultze I have reasons to believe that this is not a true refractory period or state of inexcitability. The reduced excitability is best shown in the hearts which pulsate with a slow rhythm, so that the diastolic pause is of several seconds' duration. Even with direct observation there is no mistaking the fact that an induced shock which produces a beat when sent through the heart during the diastolic pause fails to influence the rhythm when sent through the heart at the beginning of systole.

This rhythmic variation in the excitability is therefore characteristic of all the molluscan, arthropod, and tunicate hearts examined, and as these cover a wide range of species it seems highly probable that such variation in the excitability of the cardiac tissues obtains throughout these phyla.

The conditions of the excitability during an artificially produced

beat are the same as during an automatic beat. Conditions which diminish the rate and the strength of the beats, such as injury or fatigue, tend at the same time to render the decrease in excitability attendant upon the contraction more pronounced, evidently because the processes of recovery take place much more slowly under those conditions.

The intensity of the stimulus that proves efficient during diastole and the diastolic pause but inefficient at the beginning and during systole must be determined for each preparation, as the excitability varies, not only between the hearts of different species, but also between the hearts of the same species. But in the heart of all these invertebrates a greater strength of the induced or direct current proves an efficient stimulus even when applied to the heart at the very beginning of systole. The effects appear in increased or decreased strength of contraction of the same beat, in shortening of the following diastole or in increase in the tonus, all of which shows that the heart is affected by the stimulus. The inhibitory effects of the single induced shock will be considered under a separate head. The increase in the strength of the beat produced by the stimulus sent through the heart at the beginning of systole is the ordinary motor effect, but it is not obtained with equal readiness in the hearts from the different animals. It is readily demonstrated in the crustacean heart and, on the whole, in the hearts of the gasteropods and the squid, but in Octopus and Mytilus the usual effect is a decrease instead of an increase in the strength of the beat. These reactions are illustrated in Figs. 6 to 10. The tracings in Fig. 6 are from the pulsating ventricle of Ischnochiton. The single break shock sent through the ventricle at the beginning of systole may simply increase the strength of the beat without producing any change in the tonus or the rhythm (*A*, *a*); or the increase in the strength of the beats is accompanied by a shortening of the three or four subsequent diastoles (*B*, *b*). More rarely the shortening of the following diastole appears without any change in the strength of the beat (*B*; *a*). The tracings in Fig. 7, *A*, *B*, are from the same ventricle preparation of Haliotis. A relatively weak break shock produces a slight increase in the beat together with a marked shortening of the following diastole (*A*, *a*); but by greatly increasing the strength of the stimulus a very marked contraction is superimposed on the automatic beat (*B*, *a*). The myograms *C* and *D* (Fig. 7) demonstrate the excitability of the heart during systole in a different way. The

resting ventricle of Natica exhibits the accumulative effects of two, three, or four break shocks on the beat (*C*, *b*, *c*, *d*); and the same is

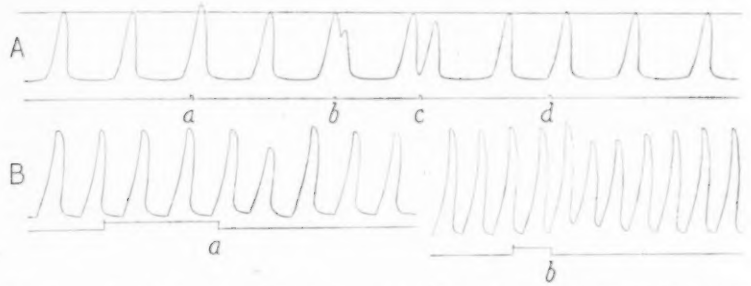

FIGURE 6. — Tracings from the beating ventricle of Ischnochiton. *A*, supermaximal beat produced by the strong induced shock sent through the ventricle at the beginning of systole (*a*). *B*, shortening of the succeeding diastolic pause by the strong induced shock during systole (*a*); a still stronger induced shock producing a supermaximal beat with subsequent augmentation of the rate (*b*).

shown in the response of the resting ventricle of Bulla to the make of the direct current (*D*, *b*, *c*, *d*). The tracing in Fig. 8, *A*, is from a

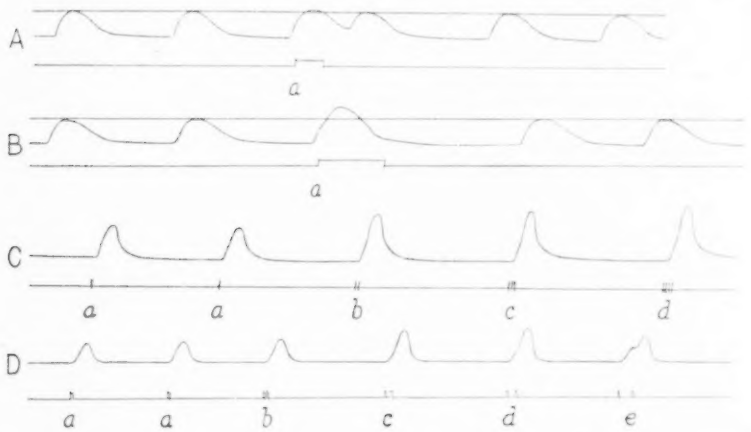

FIGURE 7. — One-half the original size. *A* and *B*, tracings from the beating ventricle of Haliotis. Supermaximal beats produced by the strong induced shock at the beginning of systole (*a*). *C*, tracing from the resting ventricle of Natica, supermaximal beats produced by strong induced shocks reaching the ventricle at the beginning of the systole of the artificially produced beat (*b*, *c*, *d*). *D*, tracing from the resting ventricle of Bulla, showing supermaximal beats produced by the make and break of the galvanic current reaching the ventricle towards the end of systole (*c*, *d*).

filled ventricle of Aplysia. It requires an induced shock of very great intensity to affect the filled Aplysia ventricle, but when such a stimulus is applied to the filled ventricle at the beginning of systole a great increase in the strength of the beat is produced, invariably followed by shortening of the succeeding diastoles (*A*). This accelerator after effect is more marked the stronger the stimulus. The same reactions are obtained from the empty ventricle of Aplysia by induced shocks of weaker strengths (*B*). A supermaximal beat is

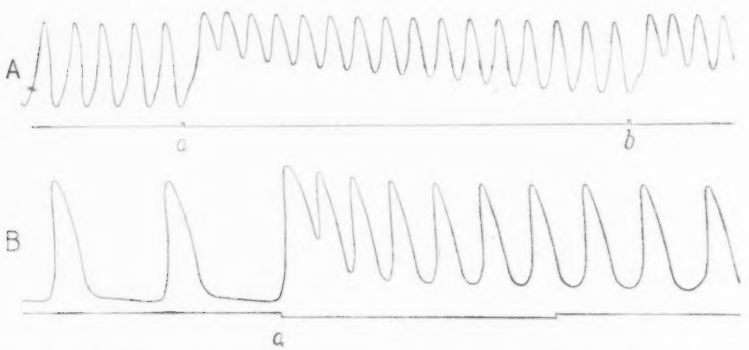

FIGURE 8. — Two-thirds the original size. Tracings from the ventricle of Aplysia. *A*, filled ventricle, supermaximal beat and tonus contraction produced by strong break induced shock at the beginning of systole (*a*, *b*). *B*, same in the empty ventricle. The induced shock in *A* is much stronger than in *B*.

readily produced by an induced shock in the hearts of the nudibranchs and the pulmonates, as will be seen by examining the tracings in Fig. 9. The tracing *A* is from the ventricle of Montereina, stimulated with the break induced shock at the beginning of systole. This myogram does not show the unusual inhibitory or injurious after-effects on the rhythm of stimulating the ventricle of this mollusc with strong induced shocks. No such injurious after-effects were ever observed in the hearts of the slugs. The usual reactions of the ventricle of Ariolimax are illustrated in tracing *B*. In this particular case the break induced shocks are used. The supermaximal beats produced by the stimuli are usually accompanied by a shortening of the subsequent diastoles. With a slightly weaker stimulus this shortening of the following diastole is less in evidence; while with a very strong induced shock this form of acceleration persists for some time, just as in the ventricle of Aplysia. The strength of the break shock is greater at *b* than at *a*.

The usual effect of a single induced shock of moderate strength applied to the crayfish ventricle at the beginning of systole is represented in Fig. 10. The supermaximal beat is followed by a marked shortening of the subsequent diastoles, the more marked the stronger the stimulus, until at a very great intensity a single induced shock is followed by a nearly complete tetanus for a few seconds. When a relatively weak shock is employed, a slight augmentation of the beat may be produced without any subsequent acceleration of the rhythm

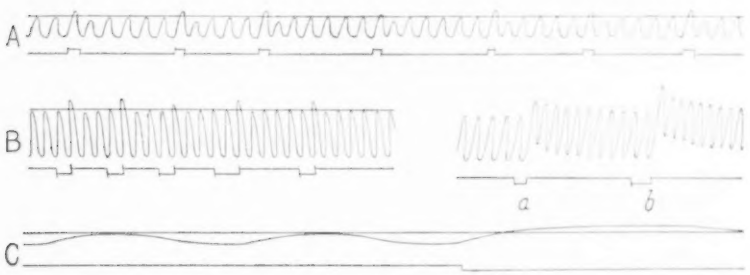

FIGURE 9. — Four-fifths the original size. *A*, tracing from the ventricle of Montereina. Supermaximal beats produced by the strong break shock (upstroke of signal) at the beginning of systole. *B*, tracing from the ventricle of Ariolimax. Supermaximal beats produced by the strong induced shock (upstroke of signal) at the beginning of systole. The strength of the stimulus is greatest in the part of the tracing to the right, and stronger at *b* than at *a*. *C*, tracing from the ventricle of Helix. Rapidly revolving drum. Supermaximal beat by strong induced shock at the beginning of systole. Showing how near the beginning of systole it was possible to stimulate the heart by the method used.

(*B*). The pulsating crab ventricle responds even more strongly to the stimulus reaching it at the beginning of systole. When an exceedingly weak stimulus is employed, the effect may be only a slight delay in the relaxation, but usually the intensity of the induced shock that affects the heart at all produces a supermaximal or a superimposed beat. These changes in excitability are equally well shown in the quiescent ventricle, that is, in an artificially produced beat.

The question whether the tunicate heart is strictly inexcitable at the beginning of systole cannot be determined on the whole heart, for the reason that all the parts of the heart are not in systole and diastole at the same time. An induced shock sent through the pulsating heart may strike the middle of the heart in systole, one end of the

heart in diastole, and the other end of the heart in the diastolic pause. But when the heart is cut into pieces of 1 or 2 mm. length, these conditions do not obtain. Pieces of the heart of Ciona usually commence a slow but regular rhythm five to ten minutes after the operation. If such an isolated and pulsating portion of the heart is observed by the aid of a lens or, still better, in the microscope, one can make out that the induced shock applied at the beginning of the contraction does not affect the beat or the subsequent rhythm unless of very great intensity; but when the very strong induced shock is employed, the diastole following the beat is shortened, and with a break shock of still greater intensity the beat fuses with a prolonged tonus (or tetanus) contraction. Any definite superposition of the beats cannot be made out. The tunicate heart is therefore not inexcitable at the beginning or during the systole, but the decrease in excitability during that phase of contraction appears to be relatively greater than in the heart of the crustaceans and the molluscs.

The statement that the hearts were stimulated at the beginning of systole is not strictly true. Between the *very* beginning of systole and the stimulation of the preparations intervened the reaction time of the observer. But this was the only method that proved applicable. A series of shocks of an intensity sufficient to affect the hearts at the beginning of systole produces tonus contraction and incomplete tetanus; and stimulating the heart with such a series with the intention that one of the shocks will at times reach the heart at the very beginning of systole gives in consequence no satisfactory results. The close approach to the beginning of systole that can be attained by breaking the primary circuit at the first sign of contraction is shown in the myogram from the ventricle of Helix in Fig. 9, *C*.

Inasmuch as these observations include animals from all of the main groups of the invertebrate phyla, we are justified in concluding that the invertebrate heart exhibits the same alterations of excitability and contractility during the different phases of the beat as the heart of vertebrates. The excitability is diminished during systole, but not abolished. That the refractory period in the vertebrate heart is not a state of absolute inexcitability, but a condition of greatly diminished excitability, is held by some of the older observers.[1] The tetanus described by so many observers for the vertebrate heart under certain conditions cannot be reconciled with a state of inexcitability

[1] TIGERSTEDT: Physiologie des Kreislaufes, 1893, p. 166.

of the heart even at the very beginning of the contraction. I have shown that a supermaximal beat may be produced in the auricle of the hagfish by a very strong induced shock sent through the auricle at the beginning of systole.[1] Porter[2] has shown that the tonus contractions of the tortoise auricle have no refractory period in the sense of absolute inexcitability. It is therefore evident that the interpretation of the refractory period of the vertebrate heart as a state of absolute inexcitability is not tenable.

So far, then, the variations in the excitability (and contractility) of the heart during the different phases of the beat are the same both for invertebrates and vertebrates. The difference is only one of degree, the vertebrate heart exhibiting on the whole a greater decrease in the excitability at the beginning and during systole than does the invertebrate heart. But the relative diminution in excitability during systole is almost as great in the tunicate heart and in the heart of some of the molluscs as in the heart of vertebrates. In the arthropods this rhythmical variation in excitability is on the whole the least marked.

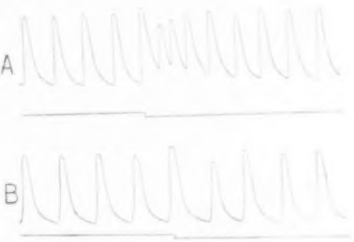

FIGURE 10. — Two-thirds the original size. Tracings from the heart of Palinurus. Super-maximal beats by induced shock at the beginning of systole. The strength of the stimulus is greater in *A* than in *B*.

On the assumption that the cause of the automatism of the heart is to be sought in the heart muscle, and that the refractory state is a phenomenon of the activity of the heart muscle, the refractory period has been brought in to explain the rhythmical activity of the heart under a constant stimulus. Now, in the first place, it has not been demonstrated that the intrinsic nervous tissue does not enter as a factor in the heart's refractory state. And even if the heart beat were myogenic and the refractory state a property of the muscle alone, the only difference this would make as regards the rhythm of hearts showing a relatively less degree of refractory condition would be the production of a more rapid rhythm, other things being equal. But there is no necessary connection, or none has so far been demonstrated, between the refractory period and the automatism or

[1] CARLSON: Zeitschrift fur allgemeine Physiologie, 1904, iv, p. 274.

[2] PORTER: This journal, 1905, xv, p. 1.

rhythmic activity of a tissue. The motor nerve fibres to the frog's skeletal muscles exhibit a refractory period of $\frac{2}{1000}$ second; while some of the motor cells in the spinal cord of the frog show a refractory period of from $\frac{1}{10}$ to $\frac{1}{20}$ second. Yet these nerve cells exhibit no greater automatism than the motor nerve fibres. Some of the pyramidal cells of the mammalian cerebral cortex exhibit refractory periods as long as $\frac{1}{12}$ to $\frac{1}{15}$ second. Yet these cells are not active automatically, at least so far as their motor functions are concerned. It will thus be seen that the relatively less diminution in excitability of the invertebrate heart during systole as compared to that of the vertebrate heart yields no support to the view that the muscle of the vertebrate heart is automatic, while that of the invertebrate heart is not,[1] it having been demonstrated for one of the invertebrates that the heart muscle does not beat automatically after the heart ganglion is removed.

[1] HOWELL: Text-book of physiology, 1905, p. 498: ERLANGER: Journal of experimental medicine, 1906, viii, p. 69.

COMPARATIVE PHYSIOLOGY OF THE INVERTEBRATE HEART. — VII. THE RELATION BETWEEN THE INTENSITY OF THE STIMULUS AND THE MAGNITUDE OF THE CONTRACTION.

By A. J. CARLSON.

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I. The Relation between the Intensity of the Stimulus and the Magnitude of the Contraction. The "All-or-None" Law.

THE experiments reported in this paper demonstrate that the so-called "all-or-none" law applies, with certain restrictions, not only to the vertebrate heart but also to the heart of invertebrates. Some light is also thrown on the mechanism by which the heart responds in this manner to direct stimulation by the discovery that the Limulus heart in good condition follows the "all-or-none" law fairly closely only as long as the ganglion is intact. This suggests that the physiological properties determining this type of response are properties of the nervous tissue in the heart rather than of the muscular tissue, or perhaps properties that depend on the normal physiological relations between the nervous and the muscular tissues. The "all-or-none" response has heretofore been interpreted by most physiologists as a peculiarity of the vertebrate heart muscle, skeletal muscle and smooth muscle not exhibiting this property. Within narrow limits the skeletal muscle responds with contraction of gradually increasing magnitude to stimuli of gradually increasing strength, while the heart responds with a maximal beat to a stimulus of minimal strength, and increasing the strength of the stimulus fails ordinarily to augment the strength of the beat.

The heart of the invertebrates has not been investigated touching this question except in the decapod crustaceans. Regarding the hearts of these animals (Homarus), Hunt, Bookman, and Tierney say: The law of "all-or-none" cannot be applied to the lobster heart; not

every contraction is maximal; on the contrary, the height of the contractions increases (to a maximum) with increasing strength of the stimuli[1] (p. 278). But their published tracings do not strictly bear out this statement. In their Fig. 7 the height of contraction remains the same while the strength of the stimulus is increased by moving the secondary coil from 15 cm. to 13 cm. from the primary, and with the secondary coil at 7 cm. distance the contraction is much weaker than the one produced by the induced shock with the secondary at a distance of 8 cm. from the primary coil. The published myograms make it very evident that these observers were working with hearts that were dying or in a poor condition.

Typical tracings illustrating the results obtained by me on the molluscan and crustacean heart are reproduced in Figs. 1 to 4. One is confronted with several difficulties in this line of experiments. The ideal preparation for the experiments is a heart which is quiescent but which retains its excitability and conductivity unimpaired; for in the pulsating heart we are confronted with the rhythmical variation in the excitability described in a previous paper,[2] and if, on the other hand, the heart is quiescent but the excitability and conductivity of the tissues much reduced, there is a possibility that the diminutive beats produced by stimuli of minimal intensities are in reality partial or local contractions, that is, maximal contractions of the musculature of a small area of the heart instead of submaximal contraction of the musculature of the whole heart. But this ideal preparation cannot be obtained, for with unimpaired excitability and conductivity of the suspended heart the tension from the weight of the recording lever is such an efficient stimulus to the rhythm that the automatic activity is kept up until the heart is exhausted. When the pulsating heart was used for the experiments, the aim was to apply the stimuli in corresponding phases of the beat, so as to stimulate the heart in as nearly identical conditions of excitability, conductivity, and contractility as possible.

The results may be summed up as follows: *The molluscan and crustacean heart that is greatly fatigued and does not beat spontaneously responds to stimuli of increasing intensities with increasing strength of contraction, but the pulsating heart or quiescent heart whose excitability and conductivity are but slightly impaired responds with a contraction*

[1] Hunt, Bookman, and Tierney: Centralblatt fur Physiologie, 1897, xi, p. 274.

[2] Carlson: This journal, 1906, xvi. p. 67.

of uniform strength to stimuli of increasing intensity within a wide range, but an increase of the intensity of the stimulus above this range is followed by beats of increased strength usually accompanied by a more or less prolonged tonus contraction. The hearts of some of the land molluscs appear to make an exception to the second part of this statement.

The myograms reproduced in Fig. 1 are typical of the response of the molluscan heart which is quiescent, fatigued, and in poor condition generally. The variation in the strength of the stimulus is indicated by the distance of the secondary coil from the primary, which is given in centimetres. No comparison should be made of the excitability of the hearts of the different animals on the basis of these figures, as the tracings were taken at widely different intervals, and different induction coils and strengths of the primary current were employed. Fig. 1, *A*, is from a completely relaxed and fatigued ventricle of Mytilus. The height of contraction increases with the strength of the induced shock, despite the incomplete relaxation after each contraction. The response of the fatigued ventricle of Mya and Cardium is very similar to that of the Mytilus ventricle; and by examining tracings *B*, *C*, and *D* it will be seen that this applies also to the "dilapidated" ventricles of the gasteropods Haliotis, Natica, and Bulla. The tracings from the empty and exhausted ventricle of Aplysia show not only the same increasing strengths of the beats, but also the marked decrease in the latent time with the increasing intensity of the break shock. The myogram *E* is from the auricle of Ariolimax. There is a gradual increase in the strength of the contractions as the secondary is moved from 9 cm. to 6 cm. from the primary coil, but only a very slight increase on moving it still closer, or from 6 cm. to 4 cm. This may be due in part to the incomplete relaxation of the auricle after the contractions produced by the strong shocks. A similar condition prevails in tracing *F* from the ventricle of Helix. The difference between the contractions at 4 and 2 is hardly perceptible, while the difference in the contractions at 10 and 8 is very great. These records are all from the heart of the lamellibranch and the gasteropod molluscs. The hearts of the cephalopod molluscs, the crustaceans, and the tunicates, even though fatigued and in very poor condition, exhibit to a much less extent these diminutive responses to weak stimuli. Unless the hearts of these animals are in a poor or dying condition their response to stimuli of increasing strength is of the second type, and will be described in that connection.

The strong induced shock produces not only a ventricular or auricular beat, but also a tonus contraction; the stronger the shock the greater and more prolonged the tonus contraction. This tonus

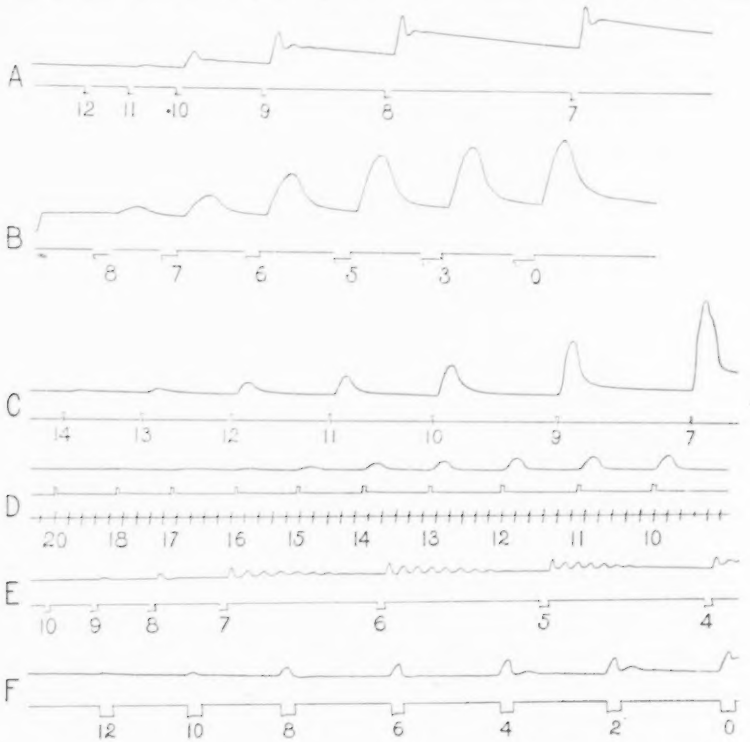

FIGURE 1. — Tracings from quiescent hearts in poor condition on direct stimulation with single induced shocks of gradually increasing strength, as shown by the distance of the secondary coil from the primary in cm. *A*, tracing from ventricle of Mytilus. *B*, tracing from the ventricle of Haliotis. *C*, tracing from the ventricle of Natica. *D*, tracing from the ventricle of Bulla. *E*, tracing from the auricle of Ariolimax. *F*, tracing from the ventricle of Helix. Showing that the molluscan heart in poor condition does not exhibit the "all-or-none" response.

reaction is more in evidence in the greatly fatigued hearts. In the less fatigued preparations the initial contraction produced by a strong induced shock is followed by a rhythmical series of beats, and it is only stimuli of very great intensity that produce tonus contractions not accompanied by diminutive beats.

Let us now consider the reactions of these hearts to stimuli of increasing strength, when the excitability and conductivity of the heart tissues are as nearly normal as possible. In the tracing in Fig. 2, *A*, the quiescent ventricle of Mytilus responds with equally strong beats at 9 and 7, and with slightly stronger contractions at 5 and 3. With the secondary coil only 5 cm. and 3 cm. from the primary, the

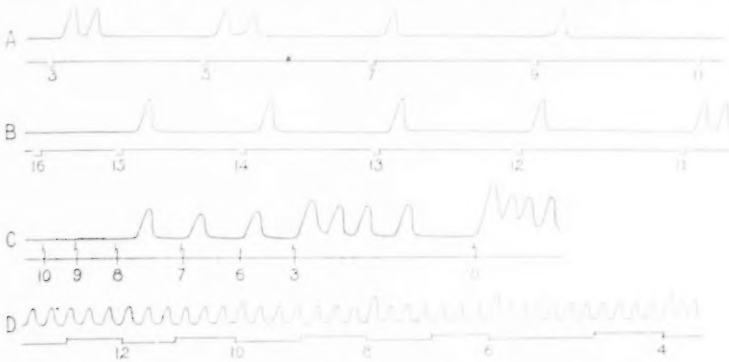

FIGURE 2. — About four-fifths the original size. Tracings from pulsating hearts or quiescent hearts in good condition on direct stimulation with induced shocks of gradually increasing intensity, the intensity being indicated by the distance of the primary from the secondary coil in cm. *A*, tracing from the ventricle of Mytilus. *B*, tracing from the ventricle of Cardium. *C*, tracing from the ventricle of Ischnochiton. *D*, tracing from the ventricle of Ariolimax. Showing (excepting *D*) a tendency to an "all-or-none" response.

break-induced shocks produced two beats instead of one. In some of the ventricle preparations of Cardium the strength of the induced shock could be increased sufficiently to produce a second or a third beat without at the same time causing any augmentation in the strength of the contractions as compared to that produced by the weaker stimuli (*B*).

The hearts of the chitons appear to possess less of this property of responding with contractions of equal strength to stimuli of increasing intensity within a wide range. This applies particularly to the ventricle of Cryptochiton. Of all the molluscs worked on, the heart of Cryptochiton showed the least tendency towards a beat of uniform amplitude. The ventricle of Ischnochiton is a better co-ordinated organ in this respect. A typical myogram from the ventricle of this mollusc is reproduced in Fig. 2, *C*. A very diminutive contraction precedes the beat at 8, the strength of the induced shock that first

affects the heart. Similar diminutive initial contractions were frequently observed in the ventricles of other gasteropods as well as in the hearts of the lamellibranchs, when minimal stimuli were employed, particularly in slightly fatigued preparations. It is difficult to say whether this slight rise of the lever prior to the strong and definite beat is due to a slight local contraction of the muscle at the poles, or whether in these conditions of the hearts a minimal stimulus produces a contraction that is characterized by such a gradually developed systole. I am inclined to think that the former interpretation is the correct one. In tracing *C* the contraction at 8 is stronger than at 7 and 6. This difference is in all probability due to the condition of the preparation, as it was frequently observed that in ventricles which had been quiescent for some little time prior to the stimulation the first beat was often slightly stronger than the succeeding ones, even when the strength of the stimulus remained the same. The beats at 6 and 7 are of equal strength, and it is of interest to note that the spontaneous beats following the strong initial contraction at 3 are of nearly the same strength as the artificially produced beats at 8, 7, and 6.

The response of the hearts of the other marine gasteropods studied resembles closely that of the heart of Ischnochiton and the lamellibranchs. We have already referred to the reactions of the fatigued and resting heart of the land molluscs. In these animals, singularly enough, the response of the fresh and pulsating heart to stimuli of increasing intensity does not differ essentially from that of the fatigued and resting heart, as will be seen by comparing Fig. 2, *D*, with Fig. 1, *E*, *F*. In other words, weak stimuli tend to produce corresponding weak contractions, and it is only in the case of the stronger stimuli that a tendency to contractions of uniform strength appears. Tracing *D* is from the ventricle of Ariolimax, the break shocks being sent through the preparation at the beginning of systole. The stimulus is first effective with the secondary at 10 cm. from the primary, but a greater increase in the strength of the beat is produced at 8, and a still greater at 6. The augmentation of the beat at 4 cm. is very nearly the same as at 6. The response of the snail ventricle is almost of the same type. The diminutive response is usually less in evidence in the ventricle of Limax, and tracings were sometimes obtained from the ventricle of this slug in which they do not appear at all.

In the response to the induced shock of increasing strength the

heart of the cephalopod molluscs resembles the vertebrate heart more closely than does the heart of any other mollusc. A typical record from the systemic ventricle of Loligo is reproduced in Fig. 3, *A*. The break shock that affects the ventricle at all produces a maximal beat at 24, and moving the secondary up to within 14 cm. from the primary — that is, increasing the strength of the stimulus from 6 to 60

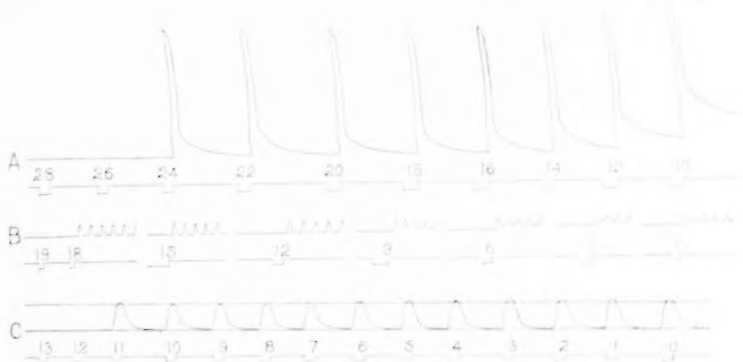

FIGURE 3. — Four-fifths the original size. Tracings from the quiescent hearts in good condition on direct stimulation with single induced shocks of gradually increasing strength, as indicated by the distance of the primary from the secondary coil in cm. *A*, tracing from the systemic ventricle of Loligo. *B*, tracing from the brachial heart of Octopus. *C*, tracing from the systemic ventricle of Octopus. Showing a tendency to an "all-or-none" response.

units — produces beats of the same amplitude; but with the secondary at 12 cm. and 10 cm. supermaximal contractions are produced, despite the fact that the ventricle is not fully relaxed. With the stimuli of still greater strength the tonus contraction is so marked that the relative strength of the fundamental contractions is obscured. The reaction of the heart of Octopus is illustrated in tracings *B* and *C*. Tracing *B* is from the branchial ventricle of a small specimen. The ventricle is quiescent but highly excitable, so that the stimulus, even the minimal, produces a series of beats. Sufficient time is allowed to elapse between each stimulation for the ventricle to return to quiescence. The break shock is first effective with the secondary coil at 18 cm., the initial beat being of the same strength as those in the subsequent spontaneous series. It may therefore properly be considered a beat of normal strength, and beats of the same amplitude are produced with the secondary at 15 cm. and at 12 cm. from the primary.

At 9 and 6 the beats are slightly supermaximal and followed by a prolonged tonus contraction, while with a still further increase in the intensity of the shock the contractions appear to be of less strength than those produced by the minimal stimuli. Part of this decrease in the magnitude of the contraction in response to the induced shock of very great strength is obviously only apparent and due to incomplete relaxation of the ventricle; but we shall presently learn that

FIGURE 4. — Four-sevenths the original size. Tracings from the heart of the crab (Cancer) on direct stimulation with single induced shocks of gradually increasing strength, as indicated by the distance of the primary coil from the secondary in cm. *A*, tracing from a quiescent heart. *B*, tracing from a pulsating heart, the heart being stimulated during the diastolic pause. Showing tendency to an "all-or-none" response.

beats of decreasing strength may be produced by stimuli of increasing intensity, even when no change in the tonus of the heart is apparent. The uniformity in the response to stimuli of a very great range in intensity is beautifully demonstrated in the systemic ventricle. In the tracing *C* the beats produced by the break shock are of the same strength, while the secondary coil is being moved from 12 to 7 cm. from the primary. At from 6 cm. up to 0 the strength of the beats is gradually increased, but the tonus of the ventricle is not affected.

The typical reactions of the crustacean (crab) heart are illustrated in Fig. 4. Record *A* is from a resting heart whose excitability and conductivity are but slightly impaired. A beat of normal strength is produced with the secondary at a distance of 23 cm. from the primary coil, and this strength of the beat is maintained while the coil is being moved up to within 13 cm., that is, increasing the strength of the stimulus approximately ten times. With the secondary at 12 cm. a supermaximal beat is produced, while the still stronger shocks at 11 cm. to 6 cm. produce an initial beat of greater amplitude followed by a rapid series of beats that virtually constitute an incomplete tetanus. By close inspection it will be seen that the beat produced

by the minimal stimulus at 23 is slightly stronger than the succeeding beats evoked by the stronger stimuli. This is of usual occurrence in the resting heart; the explanation is, in all probability, the same as that given for the same reaction in the resting ventricle of Ischnochiton. By comparing tracing *A* with tracing *B* it will be seen that the reaction of the pulsating heart is the same as that of the quiescent heart in fresh and uninjured condition. In *B* the heart is beating with a slow rhythm, and the break shocks are sent through the heart during the diastolic pause. In such preparations the amplitude and duration of the contractions artificially produced are readily compared with those of the normal beats. The break shock becomes efficient at 17, and the beat produced is normal. This amplitude of the contraction is maintained while the secondary is being moved up to within 14 cm. from the primary, but at 13 cm., 12 cm., and 11 cm. supermaximal beats are produced. The strength of the supermaximal beats at 13, 12, and 11 is very nearly uniform, but in many preparations this second "plateau" is much less in evidence. The reactions of the heart of the crayfish (Palinurus) do not differ from those of the crab heart. When the heart of the crayfish is fatigued and in a very poor condition, it responds more readily than the crab heart with diminutive contractions to minimal stimuli, described by Hunt, etc., as the normal response of the heart of Homarus.

The results obtained on the Limulus heart have been reported in a previous paper,[1] but it may be referred to briefly in this connection, because of the clue they offer to the probable mechanisms involved in the reaction of the heart to direct stimulation. Of all the invertebrates studied, the heart of the pulmonates and the heart of Limulus exhibit perhaps the least of this tendency of a maximum response to a minimum stimuli, but in the case of Limulus *the heart with the nerve cord or ganglion intact exhibits a greater excitability and a greater tendency to contractions of uniform strength in response to stimuli of varying intensities than does the same heart after the nerve cord has been extirpated.* There is a further difference that the induced shock, however strong, produces only a single contraction in the ganglion free heart, while in the intact but quiescent heart it may produce a series of beats, just as in the intact heart of other invertebrates. Whether the tendency to the "all-or-none" response depends on the direct stimulation of the ganglion and the intact nerve plexus or on a tonus condition of the heart muscle dependent on the intact ganglion, cannot be

[1] CARLSON: This journal, 1905, xii, p. 492.

determined from the data at hand. But it seems to me we have no good reason for holding that the mechanism in the Limulus heart is different from that of any other heart.

The study of a problem involving exact measurements of the amplitude of contraction is obviously attended with unusual difficulties in the tunicate heart, because of the inapplicability of the graphic method. I endeavored to measure the strength of the contraction in the heart of these animals by aid of the microscope and an eyepiece micrometer. The isolated heart of Ciona was placed in a cup made by a mould of paraffin on a slide and filled with sea water. The cup was made of sufficient depth, so that the cover glass did not press on the heart. The poles of the electrodes were brought in contact with the liquid at opposite ends or at opposite sides of the heart. By so arranging this preparation on the stage of the microscope that the decrease in the diameter of the heart tube during contraction appears as displacement of either side of the heart along the scale of the eyepiece micrometer, the displacement of the edges of the heart during the passage of the contraction wave can be determined with an accuracy unapproached by the unaided eye. In the course of the experiments it soon became evident that the question cannot be determined on the whole heart. When the heart is stimulated with a weak induced shock, the beat produced almost invariably starts at either end of the heart and travels towards the opposite end, just as the automatic beat; but when an induced shock of very great strength is employed the whole heart contracts at the same time, and it becomes impossible to tell how much of the increased displacement of the heart along the micrometer scale is due to the movement of the whole heart caused by this simultaneous contraction, and how much, if any, is due to a greater contraction of the individual muscular elements. This error is excluded by cutting the heart into small segments. Both quiescent and pulsating pieces of the heart were tested, the latter giving an opportunity of comparing the artificially produced beat with the normal automatic beat.

In its reaction to the induced shock of varying intensity the tunicate heart resembles closely the hearts of the Crustaceans and the cephalopod molluscs, that is, *the heart when in good condition responds with a maximal normal beat to stimuli from the minimal up to a very great intensity, but the induced shock of the greatest strength produces a supermaximal beat and prolonged tonus contractions.* This is illustrated by the following protocol: "July 10, 1903. Piece of *Ciona* heart, pulsat-

ing at the rate of 8 per min. Displacement of one side of heart along micrometer scale during normal systole: 95 division. Break shock effective with secondary coil at 8.1 cm. distance, heart stimulated during diastolic pause. Displacement of heart: 95, or same as in normal beat. This amount of displacement maintained while secondary moved up to within 4.5 cm. of primary. At 4 cm. displacement of heart: 120, slight tonus contraction, rhythm reduced. At 2 cm. displacement of heart: 130, strong tonus contraction lasting two minutes, no automatic beats during this interval." These slight differences could not be made out with the naked eye, but when magnified and reflected along the micrometer scale there can be no doubt about them. Single induced shocks of great intensity produce supermaximal beats. The tonus contraction is evidenced not only by the incomplete relaxation, but still more by a change in the optical properties of the heart tissue. The heart becomes much less transparent during the tonus contraction, so much so that it can hardly be accounted for by the slight increase in the thickness of the mass caused by the persistent contraction.

The diminutive beats in response to minimal stimuli observed in the hearts of the lamellibranch and gasteropod molluscs in poor condition, may be only local contractions of the muscle at the kathode or at either pole where the density of the current is greatest. In the suspended ventricles the current passed from one auricular to the other auricular end or from the auricular to the aortic end; in either case the cross-sectional area of the ventricle is the greatest in the middle. It is therefore impossible to stimulate all the parts of the ventricle with equal intensity. That the ventricular tissues of these molluscs lose the power of conduction to a great extent when extremely fatigued or when injured or otherwise in a poor condition, is really shown by mechanical stimulation. When in good condition the ventricle responds to a gentle touch with a strong and normal beat, but when in poor condition the same stimulus produces only a slight local contraction; in fact, the mechanical stimulation must be very strong in order for the contraction to extend over the whole ventricle.

The "all-or-none" type of response is ordinarily obtained in the heart of both cold-blooded and warm-blooded vertebrates when in normal condition of excitability, conductivity, and contractility. Kronecker and Mays[1] have shown, however, that when the frog's

[1] KRONECKER and MAYS: Archiv für Physiologie, 1883, p. 265.

heart is fatigued or otherwise in poor condition, the magnitude of the contraction increases with the intensity of the stimulus. That is exactly the reaction I obtained in the invertebrate heart when in poor condition. Porter[1] has recently recorded another instance of the vertebrate heart deviating from the "all-or-none" law, namely, the tonus contractions of the tortoise auricle. Porter suggests that the exceptions to the "all-or-none" response may be due to the addition of tonus to the fundamental contractions, as the tonus response varies directly with the intensity of the stimulus. The tracings published by Kronecker and Mays do not exhibit any tonus contractions. Those observers evidently secured greater amplitude of the fundamental contractions by increasing the strength of the stimulus. The fact that a very strong induced shock produces a supermaximal beat in the hagfish auricle is still another case of a vertebrate heart not following the "all-or-none" type of response.[2]

It is therefore evident that the type of response of the heart to stimuli of varying strength is the same in the vertebrates and the invertebrates. The differences that exist are only of degree, the heart in good condition of many of the invertebrates responding according to the "all-or-none" type almost as closely as the ventricle of the frog or the tortoise.

The view that the property of "all-or-none" response to direct stimulation is a property of the heart muscle apart from the intrinsic nerve plexus, is, so far as I know the literature, purely theoretical. It has not been demonstrated for any heart. On the other hand, I have shown that in the Limulus heart the "all-or-none" response depends on the intact nerve plexus in the heart. And there is anatomical basis for a similar mechanism of the "all-or-none" response in the heart of all other animals so far investigated.

II. Contractions of Diminished Amplitude in Response to Induction Shocks of Increasing Strength.

The statement that with induced shocks of a certain intensity sent through the heart the greater the intensity the greater the amplitude of the muscular response requires the following limitation. When working with the heart of crabs and the heart of Octopus, tracings were sometimes obtained showing a relation between stimulus in-

[1] Porter: This journal, 1905, xv, p. 6.

[2] Carlson: Zeitschrift für allgemeine Physiologie; 1904, iv, p. 274.

tensity and contraction amplitude, the very reverse of that just stated, *the hearts responding with beats of decreasing magnitude in response to induction shocks of increasing strengths.* A typical tracing of this type from the systemic ventricle of Octopus is reproduced in Fig. 5. In this case the make induced shocks are used and the strength is indicated by the distance of the primary coil from the secondary. The beats at 18 and 16 are of nearly the same amplitude, but when the secondary is moved up to within 14 cm. from the primary, a diminution in the strength of the beat is apparent, and the decreased amplitude becomes greater as the secondary is being moved up to 6 cm. To that strength of the shock and up to the greatest

FIGURE 5. — Four-sevenths the original size. Tracing from the quiescent ventricle (systemic) of Octopus on direct stimulation with single induced shocks (make) of different strengths. The strength of the shocks indicated by the figures, which give the distance of the primary from the secondary coil in cm. Showing diminished amplitude of contractions in response to induced shocks of increasing intensities.

the inductorium can yield the ventricle responds with a uniform but very diminutive beat. That the decrease in the amplitude of the contractions is not due to decrease in the excitability of ventricle from injurious effects of the strong stimuli is shown by the right-hand side of the record, which shows the same reactions although the series is begun with the make shock of the highest intensity. The ventricle has lost some of its excitability during the experiment, for the minimal stimulus at the end is stronger than at the beginning (16, 18). But all the preparations did not show a similar decrease in the excitability after the stimulation with the strong induced shocks. The very reversed effect was sometimes observed.

In some cases a second rise in the amplitude of the beats followed the increase of the stimulus intensity above that which produced a marked diminution. This was always the case in the heart of the crab, but in the Octopus ventricle I frequently failed to obtain it even with the strongest induction shocks available as indicated in the tracing in Fig. 5.

This peculiar action was obtained only in the crab heart and the Octopus heart, and as a rule only in fatigued preparations. The reactions are more readily obtained by the make than by the break induced shock.

The mechanism by which these depressor effects are produced is obscured by the fact that I failed to obtain them in all preparations. It may be a case of transient paralysis of the heart tissues from over-stimulation, or a condition of anelectrotonus. Further studies are required to settle this point. Similar reactions have recently been described in the case of human skeletal muscle (and nerve) by Storey.[1]

III. The "Staircase" Contractions of the Crustacean Heart.

The well-known "staircase" phenomenon, or the response of the muscle with contractions of gradually increasing amplitude to a series of induced shocks of the same intensity, has been found to obtain not only in the heart, but also in the skeletal muscle of the vertebrates. Richet obtained the same response in the claw muscles of the decapod crustaceans. Reactions which appear to be of the same nature were obtained by me in the heart of the crabs. If the resting or pulsating crab heart is stimulated with make or break shocks of uniform but not too great intensity at the rate of one stimulation per second, the contractions in response to the first three or four stimuli are of gradually increasing amplitude. The contractions following the succeeding stimuli remain of uniform strength. If the rate of stimulation is less than one every other second, no staircase effects are obtained. This successive increase in the strength of the beats appears the more singular because, when the rate of stimulation is one second, the heart is being stimulated with the second shock before it has completely relaxed from the contraction produced by the first, and from the fact of rhythmical variation in the excitability of the heart tissues one would have expected that the response to the second shock should have been less than to the first.

Various explanations of the "staircase" response in muscle have been offered. The most probable explanation appears to be that the electrical conductivity of the muscle is increased by the first few stimulations, and in consequence each succeeding shock is in reality a stimulus of greater intensity. In the case of the heart it has also been suggested that these effects are due to fatigue or gradually diminishing influence of the inhibitory nerves which are stimulated simultaneously with the muscle and the motor nervous tissue. This explanation, of course, does not apply to the skeletal muscle to which

[1] Storey: This journal, 1903, viii, p. 435.

no inhibitory nerves have as yet been found. In the crustacean heart there is a close resemblance between the "staircase" response to a series of induced shocks, the accelerator effects of the weak interrupted current and the escape of the heart from the inhibition produced by direct stimulation of the heart with a weak interrupted current. These accelerator and inhibitory effects of the weak interrupted current will be considered more fully in another connection. The resemblance between the "staircase" response and the escape of the heart from the inhibitory influence is particularly close. The initial submaximal beats in the latter case can hardly be due to increasing effectiveness of the stimulus caused by increased electrical conductivity of the muscle. It seems to me that the more probable explanation is that they are due to gradual decrease of the inhibitory influence. But the similarity of the myograms does not prove that we have to do with the same causes in the two reactions.

COMPARATIVE PHYSIOLOGY OF THE INVERTEBRATE HEART.—VIII. THE INHIBITORY EFFECTS OF THE SINGLE INDUCED SHOCK.

By A. J. CARLSON.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

I. The Inhibitory Effects of the Single Induced Shock on the Fundamental Contractions.

IN the course of the experiments on the rhythmical variations of excitability in the invertebrate heart some rather singular results were obtained, which on further examination proved to be characteristic of all the molluscan and arthropod hearts worked on. I found, namely, *that the induced shock of a certain intensity sent through the pulsating heart at the beginning of systole diminishes the strength of the beat already in progress without necessarily affecting the tonus of the heart or the strength and rate of the following beats.* But usually the strength of the shock required to produce this decrease in the amplitude of the beat causes also shortening of the succeeding diastole or diastoles together with a tonus contraction, and more rarely a lengthening of the following diastole accompanied by tonus relaxation. That the interrupted current of a certain intensity inhibits the heart of many vertebrates on direct application is well known. Foster and Dew-Smith,[1] and Ransom [2] have shown that this is also true for the heart of several molluscs. Foster and Dew-Smith concluded that the single induced shock of an intensity below that which stimulated the snail's heart produced the opposite effect or inhibition; but their demonstration of the inhibitory effect of the single induced shock is not convincing. Ransom concludes that "the only action of the single induced shock on the heart (snail, Octopus) is to excite to contraction" (p. 336). This conclusion of Ransom is certainly untenable

[1] Foster and Dew-Smith: Proceedings of the Royal Society, 1875, xxiii, p. 318.

[2] Ransom: Journal of physiology, 1884, v, p. 261.

for the heart of those invertebrates worked on by him, as well as for the hearts of all the invertebrates included in this work.

A few typical tracings showing this type of inhibition in the heart of various invertebrates are reproduced in Figs. 1 and 2.

Tracing *A* (Fig. 1) is from the ventricle of Mytilus. Both the make (up stroke of signal) and the break shock diminish the beat, the former more than the latter, but the shortening of the following diastole is more marked at the break shock. The tracing in *B* is from the ventricle of Ischnochiton. With the strength and direction of the shocks employed in this case the make shock (up stroke of signal) produces the greater decrease in the beat. These inhibitory effects are very readily obtained in the ventricles of the slugs and the snail; in fact, it was in the ventricle of Ariolimax that I first observed this reaction. The myogram reproduced in Fig. 1, *C*, is from the ventricle of this slug. The make shocks are employed, and the direction of the induced current is with the kathode on the auricular end of the ventricle. The amplitude of the beats reaches in some cases only about one-half that of the normal beats, but the tonus or subsequent rhythm is not affected. Tracings *D* and *E* illustrate the same effects of the single induced shock in the ventricle of Limax and Helix respectively. In these instances the recording surface is moving with greater speed than in tracing *C*, so that the point of stimulation (the beginning of systole) is clearly shown. Because of the lack of material, the heart of Loligo was not examined in this regard, but the reaction was obtained in the systemic ventricle of Octopus. The crustacean heart forms no exception, as is shown in the tracings reproduced in Fig. 1, *F* and *G*, from the ventricle of the crab and the crayfish.

The tracings in Fig. 2 are from the filled ventricle of Aplysia. In *A* the make shock sent through the heart at the beginning of systole not only diminishes that beat, but almost entirely suppresses the subsequent beat. In tracing *C* the make shock diminishes the beat without producing any change in tonus or the subsequent fundamental beats, while the stronger break shock in *B* produces a tonus contraction in addition to the diminution in the amplitude of that beat.

The diminution in the amplitude of the beats is rarely followed by any inhibitory after effects. These were, however, sometimes obtained in the filled ventricle of Aplysia (Fig. 2, *A*) and in the ventricles of Helix and Limax (Fig. 1, *D*). Only in very exceptional cases are inhibitory effects produced by the single induced shock sent through the heart during diastole and the diastolic pause. The shock that

affects the heart in these phases of the fundamental contraction produces an extra beat. But I have occasionally obtained a prolongation of the diastolic pause and a diminution in the amplitude of the subsequent beat by sending an induced shock through the heart

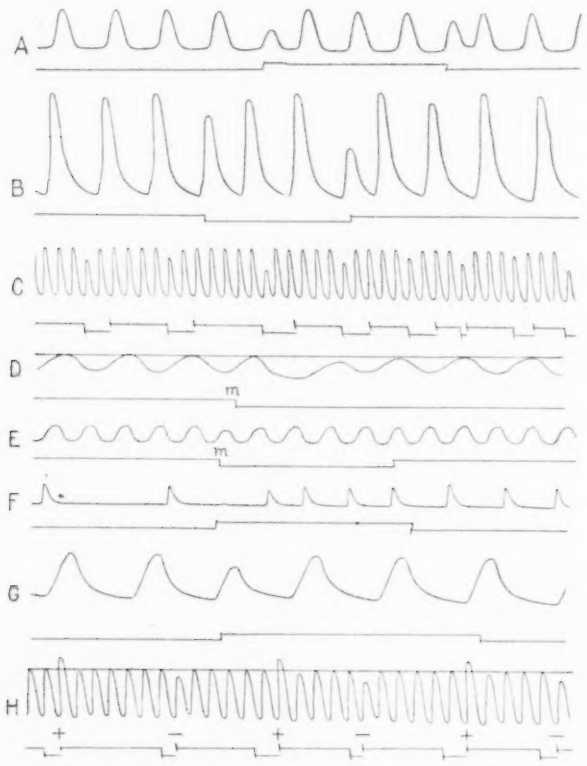

FIGURE 1. — Tracings showing diminution in the amplitude of the automatic beat on sending a single induced shock through the heart at the beginning of systole. *A*, tracing from ventricle of Mytilus, up stroke of signal = make. *B*, tracing from the ventricle of Ischnochiton, up stroke = make. *C*, tracing from the ventricle of Ariolimax, down stroke = make. *D*, tracing from the ventricle of Limax, make shock. *E*, tracing from the ventricle of Helix, stimulation by make shock (down stroke). *F*, tracing from the ventricle of the Crab (Epialtus), stimulation at up stroke (make). *G*, tracing from the heart of Palinurus, stimulation at up stroke of signal (make). *H*, tracing from the ventricle of Ariolimax stimulated with make induced shocks at the beginning of systole. Direction of current through the long axis of the ventricle +, anode at auricular end —, kathode at auricular end.

during diastole, particularly in the case of the heart of Aplysia and Ariolimax.

It is difficult to establish the intensity of the induced shock which invariably will produce diminution of the beat when sent through the pulsating preparation at the beginning of systole, because of the rhythmical variation in the excitability as well as the variation of the excitability from one preparation to another. With the method employed it is, of course, impossible to stimulate the heart successively in exactly the same phase of contraction. But on the whole the intensity of the induced shock must be relatively great and, within limits, the greater the strength the greater the degree of inhibition,

FIGURE 2. — One-half the original size. Tracings from the filled ventricle of Aplysia showing inhibitory effects of the single induced shock on the fundamental contraction. Stimulation at the beginning of systole.

Fig. 4, *B*) to produce the diminution of the beat, while a very strong induced shock applied to the heart at the beginning of systole usually produces a supermaximal instead of a submaximal beat.

The intensity of the induced shock which produces a diminution of the beat when sent through the heart at the beginning of systole produces an extra contraction when applied to the heart towards the end of the systole or during the diastole and the diastolic pause. This is very readily demonstrated in the ventricle of Ariolimax, a typical record from which is reproduced in Fig. 3. The intensity and direction of the make shocks remain the same. At *a* the ventricle is stimulated at the beginning of systole, and the result is the usual diminution of the beat and shortening of the succeeding diastole; but at *b*, *c*, and *d*, where the shock passes through the ventricle at successively later stages of the systole, supermaximal beats are produced. At *b* the systolic curve of the supermaximal beats does not differ from the normal, but at *c* and *d* the break in the curve where the artificially produced beat is superimposed can be made out. At the end of systole and during diastole the stimulus produces distinct extra contractions (*e*, *f*, *g*).

We have seen that this form of inhibition is produced both by the

make and the break shock, but in the ventricle of Ariolimax it is more readily obtained by the make than by the break, and that appears also to be true of the systemic ventricle of Octopus. In the ventricle of Ariolimax, moreover, the effect appears to depend to some extent on the direction of the current. The same intensity of the make shock which produces a diminution of the beat when the kathode is on the auricular end of the ventricle produces a supermaximal beat when the anode is on the auricular end. This is illustrated in Fig. 1, *H*. The strength of the make shock remains the same, but the direction is reversed by means of a Pohl's commutator. In this respect the influence of the induced shock is closely paralleled by that of the direct current, for in the ventricle of this mollusc arrest of the rhythm in diastole is most readily obtained with the kathode on the auricular end, while with the poles reversed the same strength of the current tends to produce a continuous contraction. But even in the ventricle of Ariolimax the diminution of the beat can be obtained by the induced shock with the anode on the auricular end.

A certain intensity of the single induced shock diminishes the amplitude of the contraction when sent through the heart at the beginning of the systole of an artificially produced beat. This is illustrated by the tracing from the quiescent ventricle (systemic) of Octopus in Fig. 4, *A*. In this case, with the secondary coil at a distance of 12 cm. from the primary, the ventricle responds with a maximal beat both to the make and the break shock, but in the part of the record represented in this figure the secondary is moved up to within 6 cm. of the primary; that is, the strength of the shocks is many times greater than suffices to produce the maximal response, but not great enough to produce the submaximal response described in a previous paper. When the make and the break shocks follow each other in rapid succession, the

FIGURE 3. — Seven-twelfths the original size. Tracing from the ventricle of Ariolimax, showing the difference in the effects of the single induced shock depending on the phase of the automatic beat in which the shock is sent through the heart. The stimulus is the make shock (down stroke) of uniform intensity. Time = seconds.

resulting beat is of less amplitude than the beats produced by each shock separately; that is to say, a second stimulus reaching the ventricle at the beginning of the contraction produced by a previous stimulus prevents the full development of that contraction.

II. The Inhibitory Effects of the Single Induced Shock on the Tonus.

The hearts of the lamellibranch and the gasteropod molluscs pass very readily into a condition of increased or extreme tonus, lasting for the longer time the more extensive the injury or the stronger the

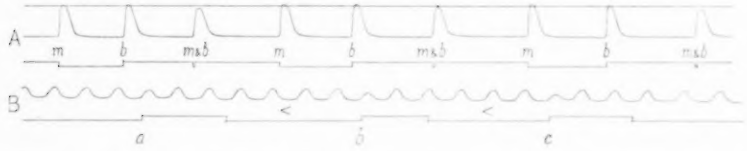

FIGURE 4. — About two-thirds the original size. *A*, tracing from the quiescent ventricle (systemic) of Octopus stimulated with single induced shocks. The make (*m*) and break (*b*) shocks produce beats of equal strength. When the break shock is sent through the ventricle at the beginning of the systole of beat caused by the make, the beats are submaximal. *B*, tracing from the ventricle of Mytilus stimulated with the make induced shock (up stroke) at the beginning of systole. The induced shock is stronger at *b* than at *a* and stronger at *c* than at *b*, showing, within limits, greater degree of inhibition with the stronger induced shock.

stimulus causing the contraction. A sufficiently strong mechanical stimulation, a strong tetanizing current, and even a single induced shock will produce this prolonged contraction. The filled ventricle of Aplysia goes into extreme tonus if the pressure of the liquid is raised above a certain maximum. In the largest and strongest ventricles the column of blood sometimes reached the height of 20 cm. before this tonus reaction was obtained. The tonus contraction is less readily produced in the ventricles of Octopus, although a very strong tetanizing current usually leaves the heart in a state of extreme tonus or "tetanus" for some seconds or even minutes after the stimulation. In the hearts of the squid and the decapod crustaceans this prolonged tonus contraction was never observed. When the contraction is extreme, no beats appear on the curve, but in cases where the heart passes gradually into the state of extreme contraction diminutive beats appear on the rising curve, and as the relaxation sets in these diminutive beats usually reappear.

In the hearts of the lamellibranchs and the gasteropods as well as in the ventricles of Octopus in this condition of increased tonus or prolonged contraction the single induced shock of a certain intensity causes relaxation of the contraction, but an increase of the strength of the shock above this intensity produces contraction or further increase in the tonus.

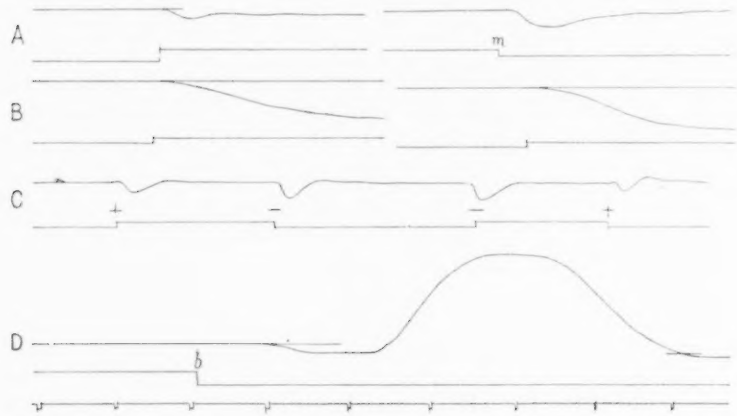

FIGURE 5. — Tracings from the quiescent heart in relatively strong tonus, stimulated with strong induced shocks, showing inhibition of tonus by the strong induced shock. *A*, tracing from the ventricle of Lucapina. *B*, tracing from the ventricle of Ariolimax, make shocks. *C*, tracing from the filled ventricle of Aplysia. Up stroke = make shock, direction of current +, anode on auricular end −, kathode on auricular end. Showing greater relaxation when the kathode is on the auricular end of the ventricle. *D*, tracing from the ventricle of Mya, the break shock producing a preliminary tonus relaxation followed by a beat.

Typical myograms illustrating this tonus relaxation in the molluscan heart are reproduced in Fig. 5. The tonus relaxation is usually only temporary, the hearts returning more or less gradually to their former degree of contraction. But if the prolonged contraction is extreme or nearly so, the recovery is usually incomplete. It is not strictly correct to call the prolonged contraction extreme, except in the case of the filled ventricle of Aplysia, for the interrupted current or even the single induced shock when of sufficient intensity will produce additional contractions. In the resting and to all appearance relaxed ventricle of Mya the induced shock of a moderate intensity usually produces a tonus relaxation prior to the contraction (Fig. 5, *D*).

The tonus relaxation is produced both by the make and the break shocks, and it is produced with either direction of the current through

the ventricle, but in the ventricles of Ariolimax and Aplysia the relaxation is usually more marked with the kathode on the auricular end. This is illustrated in Fig. 5, *C*, from the filled ventricle of Aplysia. In this particular case the extreme contraction was induced by stimulating the ventricle for a few seconds with a strong direct current. Both the make and the break shocks are employed, but their direction is reversed. By close inspection of the myogram it will be seen that both the make and the break shocks produce a slight initial contraction when the anode is on the auricular end. This contraction is wanting and the tonus relaxation is greater when the kathode is on the auricular end. These differences in the response of the ventricle are apparently dependent on the direction of the current.

The latent period of the relaxation is, with the exception of that of the filled ventricle of Aplysia, much greater than that of the contraction. In the ventricle of Mya the latent time of tonus relaxation varies from 0.5 to 1 sec. On the ventricle of Limax it is sometimes as short as 0.1 sec.; in the ventricle of Ariolimax it is usually about 0.5 sec. Those figures are considerably greater than the actual latent time, because the lightness of the recording lever does not allow a prompt descent. The latent time of contraction of the heart of these animals may be as short as 0.02 sec. In the extremely contracted (filled) ventricle of Aplysia the latent time of the relaxation does not appear to be greater than that of contraction, the latent period of both processes being less than 0.04 sec.

The tonus relaxation produced by the induced shock is sometimes preceded by a diminutive contraction. It is possible that this is invariably the case, and that the contractions are usually too diminutive to be observed or to affect the lever. If this is a correct interpretation, the tonus relaxation may be an indirect rather than a direct effect of the stimulation; that is, the relaxation may be the diastole of the diminutive contraction produced by the stimulation, as is obviously the case in many of the records.

The strength of the induced shock required to produce the relaxation is relatively great, but the excitability of the heart in this condition is lower than when the hearts are pulsating rhythmically.

These inhibitory effects of the induced shock on the tonus were not obtained in the hearts of the squid, the decapod crustaceans, and the tunicates. In the latter group the question is difficult to determine. The work on the squid heart in this line was not extensive.

III. Theoretical Considerations.

I have shown in a previous paper that this diminution of the amplitude of the automatic beat by the strong induced shock sent through the heart at the beginning of systole is also obtained in the vertebrate heart (the hagfish).[1] In this respect, then, there is no difference between the vertebrate and the invertebrate heart.

I have as yet made but little progress in determining the nature of this type of inhibition. The simplest explanation would be that it is brought about by the stimulation of inhibitory nerves and nerve endings in the heart by the induced shock. This explanation is rendered improbable by the following facts, namely: 1. The inhibition is obtained in hearts and parts of hearts in which inhibitory nervous mechanisms have not as yet been found. 2. It is obtained in hearts provided with inhibitory nervous mechanisms after these have been paralyzed by drugs (atropin, nicotin, curare). 3. The intensity of the induced shock required to produce this type of inhibition is so great as to make it highly probable that it affects the automatic heart tissue directly.

The facts already referred to that this inhibition is usually more readily obtained by the make than by the break shock, and that the efficiency to produce the inhibition appears to depend, in some hearts at least, on the direction of the induced current in the heart, suggests that we may have to do with an electro-tonic action of the induced shock. The inhibition may be only a case of anelectrotonus. It is well known that in the case of the strong galvanic current the anelectrotonic condition extends over the greater part of the inter-polar region in the tissues. This explanation is suggested by the fact that this type of inhibition requires a relatively great intensity of the induced shock. Attempts were made to test this explanation in the ventricle of the hagfish, but the results so far have not supported this explanation. The inhibition is probably not a case of anelectrotonus, or at any rate the polarizing action of the induced shock in the heart is complicated by the complicated structure of the heart (nervous and muscular tissues), and by the further fact that all the parts of the heart in automatic activity are not in the same state of contraction or relaxation at the same time. It was pointed out that the induced shock applied to the heart in diastole produces contraction, while reaching the heart at the beginning of

[1] Carlson: Zeitschrift für allgemeine Physiologie, 1904, iv, p. 259.

systole it inhibits the contractions. We thus have qualitatively different effects from the induced shock in the two conditions of the heart. The question whether this inhibition has anything in common with the inhibition of the heart by stimulation of the inhibitory nerves, and whether the induced shock produces the inhibition by acting on the nervous or on the muscular tissues in the heart will be considered in connection with the inhibitory effects of the interrupted current applied directly to the heart.

THE EFFECTS OF INTRAVENOUS INJECTIONS OF EXTRACTS OF THE BONE MARROW OF SWINE ON THE BLOOD PRESSURE IN DOGS.

By ORVILLE HARRY BROWN and DON R. JOSEPH.

[*From the Physiological Department of the St. Louis University.*]

BROWN and Guthrie[1] found that a sodium chloride solution extract of beef bone marrow produced a marked and prolonged fall of arterial blood pressure when injected intravenously. A second injection produced as great a fall as the first, but the duration of the fall was much shorter. The decreased tension is a result of vaso-dilatation, as the same fall occurs after cutting of the vagi nerves. The heart rate is even slightly accelerated during the fall.

Most tissue extracts when injected intravenously alter the height of blood pressure. Oliver and Schäfer[2] found that an extract of the medullary substance of the suprarenal capsule when injected intravenously into an animal produced a marked though transitory rise of arterial tension. These same workers[3] found that extracts of the pituitary body contained both a pressor and a depressor substance. Subsequent investigations[4] have shown that the hypophysial portion of the gland is void of either of these substances, and that both reside in the infundibular lobe. Schäfer and Moore[5] found that extracts of nerve tissue produce a fall of the arterial tension. This occurs after the division of the vagi and after atropin, so it must be a vasomotor effect. Extracts[6] of thyroid, parotid, and submaxillary glands produce a fall in blood pressure. There is present in saline

[1] Brown and Guthrie: This journal, 1905, xiv, p. 328.

[2] Oliver and Schäfer: Journal of physiology, 1894, xvi, p. 1.

[3] *Ibid.*, 1895, xviii, p. 276.

[4] Schäfer and Vincent: Journal of physiology, 1899, xxv, p. 87; Howell: Journal of experimental medicine, 1898, iii, p. 245.

[5] Schäfer and Moore: Journal of physiology, 1896, xx, p. 26.

[6] Oliver and Schäfer: Journal of physiology, 1895, xviii, p. 277.

extracts of lung,[1] liver, kidney, muscle, ovaries, testicle,[2] pancreas, and spleen, a depressor substance which is more soluble in hot than in cold salt solutions. A pressor substance, more soluble in cold saline solutions than the depressor substance, has also been found in the liver, spleen, and kidney.

The function of this depressor substance so generally present in all tissues can only be conjectured. Formerly such substances were spoken of as internal secretions because it was supposed that they had an important function in the animal economy. While it is entirely possible that these are truly internal secretions, — the pressor substance being secreted when the arterial tension is too low, and the depressor when it is too high, — it is more likely that the function of altering the height of the blood pressure is merely an accidental property, of at least some of the substances. The pressor may really be an internal secretion, elaborated for the purpose of counteracting the effects of substances which have a depressor influence upon the blood vascular system, and which are generally present as intermediary products of metabolism, — either anabolic or katabolic, or both.

Methods.

The extracts used were prepared from marrow of the long bones of swine:

1. Fresh marrow was warmed to about 40° or 45° C., or until the fat had become liquefied; the fat was poured off, and a measured amount of 0.9 per cent sodium chloride solution was added to the solid residue and kept at about 40° to 45° for a half-hour, after which the mixture was filtered through cotton. This extract, which will be termed *cold extract* hereafter in this paper, was of a pale milky color and contained no appreciable amount of solid material.

2. To the residue from the *cold extract* was added a measured amount of 0.9 per cent sodium chloride solution, and the mixture was boiled from twenty to thirty minutes and then filtered through cotton. This will be termed *sediment extract.* It was of very much the same appearance as the *cold extract,* — perhaps a little darker.

3. To fresh marrow from which the fat had been removed, 0.9 per cent sodium chloride was added, and the mixture boiled from twenty to thirty minutes. The filtrate from this was called *boiled extract.*

[1] Vincent and Sheen: Journal of physiology, 1904, xxx, p. 143.

[2] Brown-Séquard: Archives de physiologie, 1901, xxvi, p. 651.

4. Ninety-five per cent alcohol was added to fresh marrow from which the fat had been removed and the mixture boiled for about the same time as in the preceding extract. After filtering through cotton the filtrate was evaporated to dryness and taken up with 0.9 per cent sodium chloride. This was termed *alcoholic extract.* It was of a brownish color and slightly cloudy.

5. A dry extract was made by boiling with distilled water and evaporating the filtrate to dryness. This was termed *dry extract.* It was used by dissolving a weighed amount in a measured quantity of 0.9 per cent sodium chloride for injection.

6. Armour & Co.'s "glycerin extract of bone marrow" was also used.

Dogs anæsthetized with ether were used for testing all the extracts. The blood pressure was recorded from the common carotid artery by a mercury manometer. The rate of pulse and respiration was determined by a pendulum connected in an electric circuit which registered the time upon the drum. Tracings of the respiration were obtained by a trachea tube and tambour. The injections, unless otherwise stated, were made by means of a burette and cannula connected with the central end of the external jugular vein. The time of injection was recorded on the drum by means of a signal magnet. Unless otherwise stated, the injections were made at approximately body temperature. For control experiments the sodium chloride solution in the same concentration and quantity as that employed in making the extracts was injected. The dosage was twenty-five cubic centimetres, unless otherwise indicated.

Experimental Results.

Cold extract. — A first injection of this solution into a dog weighing about fifteen pounds produced a marked and prolonged fall of blood pressure. The original mean pressure was 162 mm. of mercury. The fall was 112 mm., and at the end of twelve minutes the blood pressure had regained a height of 102 mm. and had ceased rising. At the moment of injection a slight rise of the arterial tension of about 5 mm. Hg occurred. This is but slightly more than the rise occurring with this amount of 0.9 per cent sodium chloride solution. The point of least pressure was reached forty-seven seconds after the end of the injection. At this time the pressure rose gradually 44 mm. and again fell to nearly the former level.

A second injection was made. The pressure was first increased 32 mm. of Hg, but the increase lasted only about thirteen seconds, at which time the blood pressure fell quite suddenly to the lowest point reached in the first injection. Following this was an increase of pressure, which rose 28 mm. higher than the normal at the time of injection. This lasted for about four and one-half minutes, after which time the pressure again fell to below the normal, and regained the normal level only after about fifteen minutes. Succeeding injections produced the same effect as the second injection.

Sediment extract. — This solution produced both pressor and depressor effects, but neither was very marked or prolonged. The latter amounted to 16 mm. of mercury and lasted for thirty-six seconds. The pressor effects which occurred during the time of injection amounted to 12 mm. Hg and lasted six seconds. The blood pressure was about 5 mm. higher after the depressor effects were over than it was before the injection. Subsequent injections produced practically the same result.

Boiled extract. — The injection of this solution produced, first, a small sudden rise of the pressure; second, a sharp and deep fall; third, a quite rapid and high rise; and, fourth, a marked and prolonged fall. The first pressor effect was 18 mm. Hg; the first fall was 28 mm., and the second rise was 44 mm. The point of lowest tension shows a fall of 82 mm. from the normal. After twelve minutes the pressure rose within 10 mm. of the normal level and ceased rising. A second injection produced the same initial rise, followed by a short fall as before. The succeeding rise, which was 22 mm. above the normal, was not followed by a fall much below normal, as occurred in the "first injections." A third injection produced greater pressor effects, and the depressor results were about the same as in the second. After cutting the vagi, both the pressor and the depressor effects occurred as before.

One experiment was done with a boiled extract prepared from ox marrow. This did not produce the initial rise, but the first fall was followed by a sudden sharp rise, which was followed by the usual marked and prolonged fall, that lasted about fifteen minutes. This rise, after the beginning of the depressor effects, was not so pronounced in Brown and Guthrie's experiment.

Alcoholic extract. — This solution produced a slight initial rise, followed by a sudden fall of 40 mm. Hg; the pressure quickly returned to the normal height and remained there. A second in-

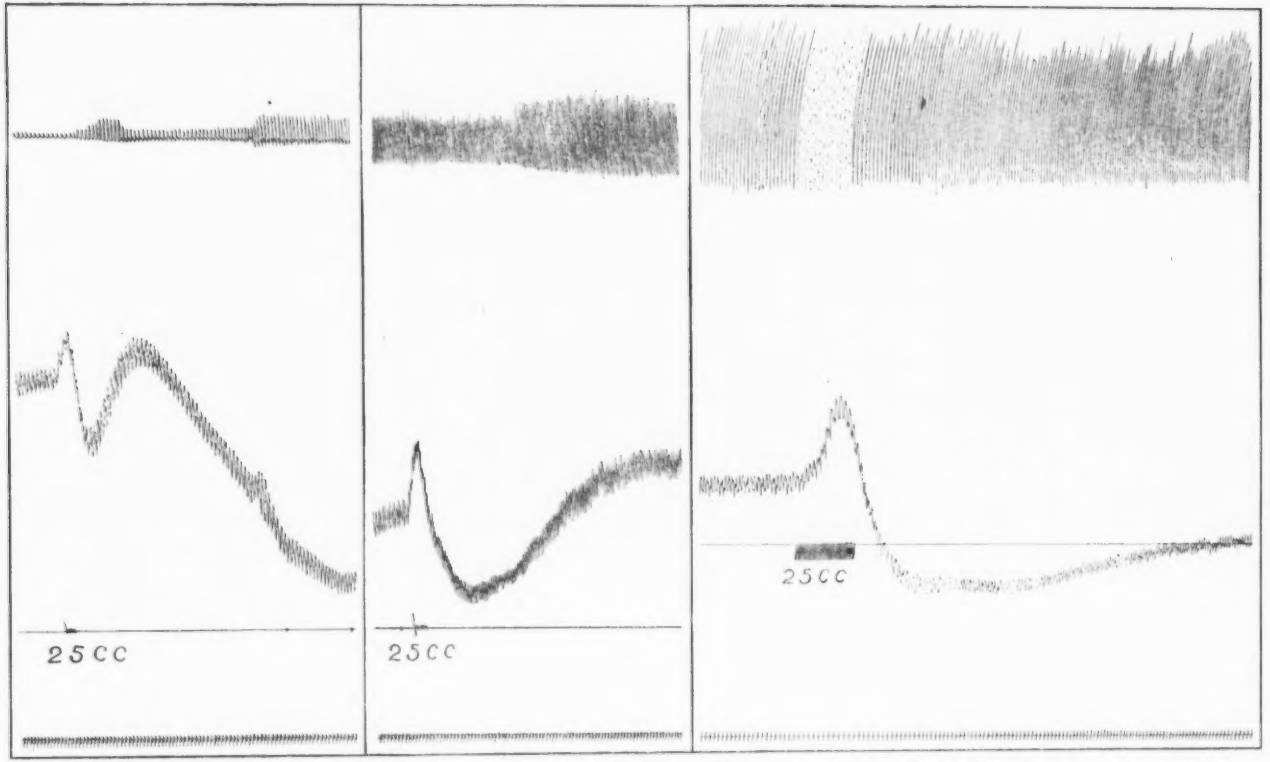

FIGURE 1. — First injection of boiled extract. FIGURE 2. — Third injection of boiled extract. FIGURE 3. — Third injection of cold extract. The tracings from above downward are those of respiration, blood pressure, injection signal magnet, and time tracing, which is also the base line for the blood pressure. The time is in two-second periods.

jection of 50 c.c. produced a sudden fall 66 mm., but the pressure returned practically to the normal. A third of 75 c.c. produced a sudden fall of 84 mm.; the arterial tension failed by 20 mm. to return to the normal after this injection.

Dry extracts. — The injection of 60 mgm. of this extract dissolved in 25 c.c. of 0.9 per cent sodium chloride solution brought the typical small initial pressor effect followed by a fall, usually occurring only after the second injections of the boiled extract. The initial pressor effect lasted a little longer than usual. 120 mgm. of the extract dissolved in 25 c.c. of 0.9 per cent sodium chloride solution produced double the pressor effect obtained with 60 mgm., and the fall was much more prolonged.

Glycerin extract. — This solution produced no initial pressor effect, but 10 c.c. produced a fall of 64 mm., which lasted about seventy seconds. Subsequent injections of the same amount produced a fall less in depth and duration. Ten cubic centimetres of pure glycerin produced practically no change in the blood pressure.

Subcutaneous injections of solutions containing active pressor and depressor substances produced very slight depressor effects, which were followed by pressor effects of 14 mm. Hg, which lasted four minutes. Administration of the solutions containing active pressor and depressor substances per stomach produced no change in blood pressure. Usually after five or six intravenous doses of the extract the animal died, and the heart was often found to be full of blood clots. Solutions made from both red and yellow marrow taken separately revealed no difference in the distribution of either the pressor or depressor substance.

Summary.

(1) The bone marrow of swine contains substances which exert pressor and depressor influences on the blood pressure of dogs.

(2) The pressor substance is soluble in 0.9 per cent sodium chloride solution at 45° C. and at boiling temperature. It is not soluble in glycerin or is destroyed by it, and is only partially soluble in alcohol or is partially destroyed by it.

(3) The depressor substance is soluble in 0.9 per cent sodium chloride solution at 45° C. and also at boiling temperature; it is soluble also in alcohol and glycerin.

(4) "The first injection" of 0.9 per cent sodium chloride solution extracts made at 45° C. produces a slight increase of blood pressure,

followed by a very marked and prolonged fall. The initial rise was not nearly so marked as that produced by later injections, while the fall was more marked both in depth and in duration.

(5) Second and later injections produced a marked but short increase of blood pressure, also a deep fall; but this fall was broken by a second pressor effect which brought the pressure again above the normal, where it remained for some time. It was replaced in from one-half to four minutes by a second depressor effect, which was gradually overcome, and the pressure gradually attained a level usually somewhat below the normal, where it remained constant.

(6) The boiled extract produced on first injection both pressor and depressor effects very similar to those obtained by "second injections" of the cold extract; the second and later injections had practically the same effects as the first injection, except that the time occupied before the pressure regained a normal level was much decreased.

(7) The effects of marrow extracts on the blood pressure can be most easily explained by the assumption that there are two separate pressor substances and one depressor substance present in the marrow. One of the pressor substances acts instantly on introduction into the blood vessels, and the other acts only after a minute or so. The depressor begins with its action immediately after the first pressor effect, and reaches its maximum effect shortly afterward, only to be overpowered by the second pressor action. This latter is spent while the depressor substance is still potent.

THE RELATION BETWEEN CONTRACTILITY AND COAGULATION OF THE COLLOIDS IN THE CTENOPHORE SWIMMING-PLATE.[1]

By RALPH S. LILLIE

Introductory and Theoretical.

At various times comparison has been made between the contraction accompanying the onset of rigor mortis in dying muscle and the normal phenomenon of vital muscular contraction. Thus, Nysten,[2] in 1811, reviving an earlier or even ancient[3] conception, characterized rigor as the last contractile effort of dying muscle. Two fundamental observations — first, that during rigor the muscle substance undergoes a change resembling coagulation, and, second, that whenever coagulation is directly induced by artificial means (as heat or the action of reagents) the muscle shortens and thickens, becoming permanently contracted — early led to the supposition that a transitory coagulation of the proteids might possibly be the immediate cause of contraction in living muscle. This view has in recent times found its most definite advocate in Hermann,[4] who called attention, in his "Handbuch" and elsewhere, to various apparently significant resemblances between the two processes. He was especially impressed by the marked similarity between the chemical changes during rigor and during prolonged normal contraction respectively; also by the existence of what appeared to be transition stages between the two conditions, such as the gradual passage of abnormal tetanic

[1] The present research was conducted at the Zoölogical Station in Naples with the aid of a grant from the Carnegie Institution. I take pleasure in expressing my best thanks to the director and staff of the station for the many courtesies extended to me during my stay.

[2] Nysten: Recherches de physiologie et de chemie pathologique, 1811 (cited from Sanderson: Schäfer's Physiology, ii, p. 390).

[3] Schiff: Gesammelte Beiträge zur Physiologie, Lausanne, 1894, ii, p. 97.

[4] Hermann: Handbuch, i, pp. 250 *et seq.*, pp. 331 *et seq.*

conditions (due to strychnine, etc.) into true rigor mortis. A muscle brought into the initial stages of rigor by ligaturing its arteries is similar to one exhausted by prolonged work in the further respect that circulation may in both instances restore the lost irritability. Schiff[1] has advocated views similar to those of Hermann. The fact has long been familiar that excessive muscular effort immediately preceding death hastens the onset of rigor; according to Schiff, it is impossible to make any rigid distinction between vital muscular contraction and the shortening of rigor; there may even be a gradual passage of the one into the other. In particular, he identified the localized or "idiomuscular" type of contraction with the contraction-phase of rigor; the latter he declared to be essentially an idiomuscular contraction produced by the irritating action of the altered sarcoplasm (Muskelsaft) of the dying muscle upon the contractile elements of the tissue.

Hermann[2] also compared experimentally the lifting power of muscle in rigor-contraction with that of normal muscle, finding that both height and lifting power of rigor-contraction may be greater than in the single muscular twitch. This is certainly not true of tetanic contraction; it is nevertheless noteworthy that a contraction due to the coagulation of the muscle-proteids can perform work quantitatively comparable with that done by the muscle in normal contraction. Brodie and Richardson,[3] in estimating the "absolute force" of heat-contraction in muscle, reached a similar conclusion; the lifting power of heat-coagulated frog's muscle was found to be equivalent to about twenty grams per square millimetre of cross-sectional area, a value again comparable with that of normal contraction.

When a muscle is coagulated, it shortens and thickens, undergoing a change of form similar to that of normal contraction. It is not, however, *a priori* clear that this coagulation is in itself the primary condition of the shortening; conceivably the latter might be a consequence of other and secondary changes accompanying coagulation. That the shortening is directly dependent on the coagulation is the generally accepted belief, and two chief facts may be adduced in

[1] SCHIFF: Gesammelte Beiträge, 1894, ii, pp. 102 *et seq.*

[2] HERMANN and WALKER: Archiv für die gesammte Physiologie, 1871, iv, p. 186; HANDBUCH, i, p. 144.

[3] BRODIE and RICHARDSON: Journal of physiology, 1897, xxi, p. 353; Philosophical transactions, 1899, cxci, p. 127.

its support: first, in the case of rigor, the characteristic stiffening or rigidity may appear independently of contraction;[1] this last does not seem to follow unless there is also a coagulation of the muscle-proteids; secondly, there exists a correspondence between the respective temperatures at which the successive contractions occur in a muscle brought into a condition of heat-rigor by gradual rise of temperature, and the temperatures at which the several constituent proteids undergo heat-coagulation.[2] The same is true of other fibrillar structures, as nerve and tendon.[3] It seems therefore clear that coagulative changes may in themselves produce contractions similar to the normal in respect both to energy-production and character of form-change. The question is whether such changes do in fact form the condition on which vital contractile processes depend. Is it possible that processes so conditioned should show the promptitude of response to stimuli and the ready reversibility that the requirements of living tissues demand?

In one respect the facts of histology are in harmony with this hypothesis. The uniform presence of a fibrillar structure in all special contractile tissues, with the direction of the fibrils coinciding with that of the contraction, is intelligible on the view that contraction is due essentially to an aggregation of the colloid constituents of the contractile elements. In a tissue composed of parallel and isolated fibrils the aggregation of the colloid particles can occur only within the individual fibrils, not between adjoining fibrils. A shortening, with a corresponding thickening, is thus the inevitable consequence of any coagulative change; while the coagulation of a tissue uniform in structure along different axes (as a piece of liver) is accompanied by a contraction in all three dimensions of space.[4] In this last case the tissue shrinks and loses water, while in the former the fluid portions need undergo only a certain displacement within the tissue.

This last consideration may aid in explaining the character of the visible changes in contracting striated muscle. It is clear that in any contraction due to an aggregation of the colloid constituents of such a tissue there must be a displacement of its more fluid portions; fluid is not lost from the tissue, since its total volume remains un-

[1] FOLIN: This journal, 1903, ix, p. 374.

[2] BRODIE and RICHARDSON: Philosophical transactions, 1899, cxci, p. 127.

[3] BRODIE and HALLIBURTON: Journal of physiology, 1904, xxxi, p. 473.

[4] BRODIE and HALLIBURTON: *Loc. cit.*

altered. If the above view is substantiated, the interchange of fluid between the isotropic and the anisotropic portions of the fibril[1] — the latter increasing in volume during contraction — must be regarded as merely a secondary consequence of the aggregation-change in the colloidal system, and not as in itself the immediate and determinative condition of the movement. It is clear that any theory which refers contraction to this interchange of fluid[2] must first explain how the interchange is effected.

If we admit the possibility that the direct source of the energy of contraction is a coagulative change in the colloids forming the contractile fibrillæ, it remains to consider somewhat more closely the nature of the conditions under which such changes can furnish a source of mechanical energy. In any colloidal solution a large portion of the transformable energy of the system exists as surface-energy in the surface — frequently of enormous extent — separating the two phases (*i. e.*, the colloid substance and the medium, or the colloid-rich and the colloid-poor phases). The measure of this potential energy is the product of the area of this surface into its specific surface-tension. One distinctive feature of any colloid system is the readiness with which the area of this surface may be made to undergo change. The action of electrolytes, changes of temperature, and mechanical disturbance[3] are the most effective agencies in causing such changes. These very agencies are known to be most effective as protoplasmic stimuli. During the aggregative changes leading toward coagulation there is a diminution of surface, and the available surface-energy decreases proportionately (assuming other conditions unchanged). A portion of the surface-energy thus transformed may appear as mechanical energy, and work is accomplished. Thus it is clear that when any colloid by coagulating brings its formerly separated particles in union and displaces the interstitial fluid, a certain mechanical work is performed. Work of at least equivalent value must be done on the system to restore the original condition.

Applying these considerations to any contractile tissue, — such a tissue, on the above view, may be regarded as essentially a device for rendering mechanically available the stored surface-energy of its constituent colloids. It is for the purpose, so to speak, of furthering

[1] ENGELMANN: Proceedings of the Royal Society, 1892, lvii, p. 411.

[2] *Cf.* PAULI'S hypothesis correlating contraction with the phenomena of swelling: Wiener klinische Wochenschrift, 1898, xi, p. 311.

[3] RAMSDEN: Zeitschrift für physikalische Chemie, 1904, xlvii, p. 336.

the accomplishment of this function that the colloid material is disposed in the form of fibrillæ. Under this condition the coalescence of adjacent particles in the fibrillæ necessitates a shortening of the latter, and the entire contractile structure accordingly shortens in the direction of the fibrillæ and broadens transversely. When the coagulative change is reversed, the original state is restored. We may suppose that if the reverse change were to proceed beyond this stage the structure would attain more than its original length. It is now known, in fact, that muscular relaxation is an active process;[1] and if contraction and relaxation are merely the reverse phases of the same process, the fact becomes in a measure intelligible on the above hypothesis.

Objections to a coagulation-theory of contraction have usually been based on the belief that coagulation is a process requiring an expenditure of time disproportionate to that occupied by a muscular contraction, and that the change is either irreversible in its nature or reversible with difficulty. Such objections have been shown by recent research to be unfounded. In any colloidal system the surface-relations between colloid substance and medium are subject to a wide range of variation either in the direction of diminution or of increase of the surface between the phases. Precipitation or actual separation of solute and solvent is with most colloids a merely incidental occurrence, taking place when the particles have attained certain critical dimensions[2] (simultaneously, as a rule, with loss or diminution of surface-charge) rendering unstable the former condition of uniform distribution throughout the solvent. Gravity, or, according to Bredig,[3] the surface-tension of the colloid-rich phase, may then effect a separation of the constituents. Re-solution, or a return to the condition of uniform distribution, will readily occur under appropriate conditions, — as the presence of active ions bearing charges of the same sign as that of the colloid. Even heat-coagulation in its incipient stages may be reversed on lowering the temperature.[4] It is evident that work must be done in opposition to the

[1] See the papers of SHERRINGTON: Journal of physiology, 1894, xvii, p. 27; Proceedings of the Royal Society, 1897, lxii, p. 183; 1899, lxiv, p. 179; 1901, lxvi, p. 66.

[2] *Cf.* BILLITZER: Sitzungsberichte der kaiserlichen Akademie der Wissenschaften, math.-naturw. Klasse, 1904, cxiii, p. 1201.

[3] BREDIG: Anorganische Fermente, Leipzig, 1901.

[4] CORIN and ANSIAUX: Bulletin de l'académie royale de Belgique, 1891, xxi, p. 345.

surface-energy of the coagulated colloid in order to restore the original state. In general, however, when coagulation is merely a manifestation of change in the state of aggregation, and is unaccompanied by alteration in chemical composition, the change is perfectly reversible. It may be said that the conditions of equilibrium in a colloidal system containing electrolytes require that there should be a certain extent of surface between colloid substance and medium. Change in the proportion or nature of the electrolytes shifts the state of equilibrium, the surface being either increased or diminished according to the conditions. The changes in the colloids of contractile tissues must be considered from this point of view.

Thus we may imagine the essential change that occurs during a muscle-twitch to be a temporary coalescence of colloid particles in the fibrillæ, due probably to alterations in the concentration (or possibly the nature) of the ions in the sarcoplasm or in the fibrillæ themselves. The entire change need occupy no more than a small fraction of a second. Its nature may be more clearly realized by the use of a familiar analogy. Imagine acid to be added rapidly to an alkaline solution of alkali-albumin; since the change in reaction must be a continuous one, there will be in any region of the solution a coalescence of colloid particles as the neutral point is approached. This incipient coagulation will be rapidly reversed as soon as the fluid acquires a definite acidity. The coagulative change may not progress sufficiently for the particles to attain visible dimensions, but it must be assumed that it occurs at a certain stage and is then reversed. In a contractile structure, like a muscle-cell or cilium, a similar temporary coalescence of particles forms, on the above view, the condition of contraction.

On this conception the occurrence of a large number of alternating contractions and relaxations in a second, as in cilia or the wing-musculature of insects, no longer remains inexplicable. If coalescence of adjacent colloid particles is conditioned by changes in their surface-tension, as Bredig's hypothesis holds,[1] a rapid alternation of

[1] Billitzer (*loc. cit.*) is disposed to reject this explanation, finding that in certain colloidal solutions stability may be greatest in the isoelectric condition (contrary to the principle enunciated by Hardy), while others (colloidal mercury) may be precipitated by electrolytes that *decrease* surface-tension as well as by those that raise it. On the other hand the fact that precipitation of an uncharged proteid colloidal solution by alcohol is hindered when the particles are given a charge, is, on Pauli's view, evidence that the flocking is largely dependent on a surface-action which is opposed by the charge on the particles. This is essentially Bredig's

greater and less degrees of surface-tension must be assumed in order to account for this phenomenon. As well known, it is possible by using as an interrupter the changes in form of a mercury-drop under electrically induced alterations in its surface-tension to make and break an electrical circuit many times in a second. Such rapid alternations of greater and less surface-tension may conceivably occur in the colloid particles composing contractile fibrils (under conditions as yet unknown), and condition a correspondingly rapid alternation of contraction and relaxation.

Evidence that contraction is accompanied by a coagulative change is afforded only indirectly by certain of the general facts of muscle physiology, while many others are difficult of interpretation on this hypothesis. It remains to examine the conditions in simpler forms of contractile tissue. The present paper describes certain observations made during a study of the relation of electrolytes to contractility in the Ctenophore swimming-plate; they seem very clearly to indicate that the above condition actually exists in this structure. The swimming-plate is substantially a cilium of macroscopic dimensions; and since ciliary activity is a remarkably widespread and evidently primitive form of contractility, the presumption is that its conditions are far simpler than in the case of muscle. The facts to be presented indicate that contraction in these structures is in fact correlated with a coagulative change in the colloids composing the contractile fibrils.

Experimental.

The following experiments were performed on the swimming-plates of Eucharis lobata, a large Ctenophore, common in the Gulf of Naples. In this form, as typically in Ctenophora, the plates are disposed serially and at almost equal intervals along eight equidistant meridional bands extending from the aboral pole of the animal to the neighborhood of the oral region. The individual plate is in reality a compound structure, as its development shows, and is composed of a large number of greatly elongated fused cilia; in Eucharis each plate may attain a length of from one to two millimetres; the width

conception. *Cf.* Pauli: Naturwissenschaftliche Rundschau, 1906, xxi. Nos. 1 and 2. That surface-action is a chief condition of the precipitation of many colloids is further indicated by the fact that shaking (*e. g.*) an As_2S_3 hydrosol to which a little dilute acid has been added enormously accelerates the separation of large flocculi. The shaking brings the particles in mutual contact more frequently than would otherwise be the case; and, once in contact, surface-action causes a coherence, etc.

is approximately the same. At rest, the plate lies flat on the surface of the row with its extremity directed orally; on contraction it gives a quick, sudden beat toward the aboral pole, followed by an immediate return to the resting position. In the normal life of the animal the plates are stimulated to beat by an impulse that passes rapidly along the row from aboral to oral, causing a beat of each successive plate as it passes. The rhythm of this movement is variable, and usually exhibits considerable irregularity according to the manner in which the impulses are discharged; from three to six beats in a second is a usual rate. Portions of a row of plates, or even single plates, may be isolated, and will show typical and often quite regular beats of approximately the above rhythm. Each plate is therefore capable of rhythmical movement independently of the impulse from the aboral region. A remarkable peculiarity of the movement is its susceptibility to inhibition by slight mechanical stimuli. Touching a row of beating plates, a detached portion of a row, or even an active individual plate with the extremity of a glass rod is typically followed by an immediate and complete cessation of movement.[1] After an interval activity is resumed.

The contraction of a plate is under normal conditions unaccompanied by any visible change in its substance, which is clear and transparent, and shows under the miscroscope evidence of its composition from fused cilia. A quick wave of contraction passes from the base of the plate, where movement starts, to its apex; ordinarily the movement is so rapid that all parts appear to contract simultaneously.

When part of a row of plates is detached from the animal, freed as far as possible from adhering sea water, and transferred to certain artificial solutions, the character of the movement undergoes marked change. The effect seen in slightly acidulated isotonic sodium bromide or sodium chloride solution[2] is typical; the rhythm is greatly accelerated, the movement becoming quick and vibratory with the individual beats usually indistinguishable; it is no longer subject to mechanical inhibition, and its energy is often greatly increased so that the strips with the attached plates are propelled rapidly through the solution. Such movement never continues long; its activity pro-

[1] This effect is seen in sea water; in many artificial solutions it is absent: the presence of calcium salts seems essential; in calcium-free sea water the reaction is greatly reduced or altogether absent; similarly in calcium-free artificial media.

[2] For a list of solutions in which this effect is seen, see p. 126.

gressively decreases and it finally ceases, usually within a minute or less, the exact duration varying in different solutions. Accompanying the movement there is seen a remarkable change in the appearance of the plate: its substance, at first clear, gradually clouds, then whitens, and finally becomes perfectly white and opaque. When this final stage is reached movement ceases and is thenceforth incapable of renewal. In other words, the abnormally increased contractile activity is accompanied by a progressive coagulation of the colloid substance of the plate. There occurs also a profound alteration in its structure and physical consistency. In the unaltered condition the plate possesses considerable coherence and tensile strength, and it is difficult to separate into portions except by teasing along the direction of the component fused cilia; its structure while living is thus typically fibrillar. After the above coagulation the coherence is found to be almost completely lost, and the entire structure is very readily broken into small fragments by simple shaking. Immediately after the coagulation the original fibrillar structure remains partly evident under the microscope, the coagulated particles exhibiting a disposition along well-defined rows corresponding to the fibrils. Later the plate absorbs water,[1] swells, loses all apparent structure, and finally disintegrates.

Various facts indicate that this peculiar coagulative change is intimately connected with the contraction itself, and is not merely due to the direct action of the solution on the coagulable substance of the plate. Thus, it frequently happens that out of a number of plates immersed simultaneously in a solution, several may begin vibrating at once, while others remain inactive for a time, — it may be for several minutes. Under such circumstances the active plates invariably show immediate coagulation, while the others remain transparent and unchanged in appearance until the vibratory movement begins in them also, when coagulation follows in the usual manner. Again, the degree of activity or energy of the movement varies in different solutions, and it is found that the rapidity with which the coagulation appears bears a direct relation to the intensity of the vibratory movement. The following illustrates: in a series of solutions of $\frac{6}{10}$ *m* ammonium chloride, containing hydrochloric acid in

[1] The degree of this swelling varies with the composition of the solution: in general, active anions (as hydroxyl, etc.) favor swelling, while cations (as hydrogen, various bivalent and trivalent metals) check or prevent the process. The relation of ions to swelling will be considered in another paper.

graded proportions, ranging from $\frac{m}{10000}$ to $\frac{m}{500}$, it is found that on passing from the solutions with lower to those with higher concentration of hydrochloric acid there is a progressive increase in the rapidity and energy of the vibratory movements and also in the proportion of plates that show such movements immediately after immersion in the solution. In the more weakly acidulated solutions movement is less energetic and lasts longer. Thus, in one series of experiments the following was observed: in isotonic ammonium chloride solution containing $\frac{5}{10000}$ *m* hydrochloric acid a large proportion of plates began active movement at once; in every instance these whitened within a few seconds; a few remained inactive and transparent for a short time, then exhibited the characteristic quick vibration, followed by immediate rapid coagulation. In solutions with somewhat more acid ($\frac{5}{10}$ *m* NH_4Cl + $\frac{7}{10000}$ *m* HCl) the initial movement was extremely active, and was exhibited by practically all the plates, many strips swimming actively during the few seconds previously to coagulation. Immediate coagulation occurred in all the active plates. In higher concentrations of hydrochloric acid ($\frac{10}{10000}$ *m*, $\frac{15}{10000}$ *m*, $\frac{20}{10000}$ *m*), the same extremely rapid vibration and coagulation within a few seconds were seen. In general, the higher the concentration of acid in such solutions (up to a certain limit), the more promptly does vibration appear, the greater is the energy of vibration, and the more rapid the coagulation of the plate.

Similar phenomena may be observed in a large number of solutions. In acidulated isotonic solutions of a number of other salts (NaCl, KCl, Na_2SO_4, $MgCl_2$, $CaCl_2$) extremely active vibratory movements with rapid coagulation appear in the solutions containing more acid (hydrochloric acid in concentrations from $\frac{m}{200}$ to $\frac{m}{800}$); in more weakly acid solutions ($\frac{m}{4000}$ hydrochloric acid or less), movement is less energetic and lasts longer, and coagulation is slower. The same is seen in acid solutions of non-electrolytes (cane sugar, dextrose, glycerine), although the movement is slower than in salt solutions. Obviously the presence of acid favors the appearance of the quick vibratory type of movement, and hence also of the accompanying coagulation. In many other cases it is difficult to see any clear relation between the composition of the solution and the production of the above effect. Alkali has a less distinct action than acid; in weakly alkaline solutions of sodium chloride (solutions of sodium chloride containing sodium hydrate in the concentrations $\frac{m}{1600}$, $\frac{m}{12800}$, and $\frac{m}{25600}$ proved most favorable) numerous instances were seen in which the plates that first became active

underwent prompt coagulation, while the inactive plates remained transparent. This phenomenon, in fact, is very frequent; some solutions in which it was observed with especial clearness are mixtures of potassium chloride and magnesium chloride (50 and 60 volumes $\frac{6}{10}$ *m* KCl + 50 and 40 volumes respectively of $\frac{6}{10}$ *m* $MgCl_2$), and of potassium chloride and calcium chloride in similar proportions. This association of coagulation with active vibration and its failure to appear, or retardation, in inactive plates are also frequently observed in pure neutral isotonic solutions of sodium chloride, potassium chloride, ammonium chloride, and calcium chloride. At times certain plates may remain permanently inactive; these later undergo coagulation, but the change is a relatively gradual one. Vibratory activity invariably hastens to a marked degree the onset of coagulation.

As already remarked, it has not been possible to discern any very definite relation (except in the case of acid) between the composition of the solutions and their effect in producing rapid vibration and coagulation. On the other hand, in many solutions in which the movement preserves from the first its normal slow rhythmical character, — such are solutions of sodium salts containing salts of various (mostly bivalent) metals in certain proportions,[1] — this rapid coagulation does not occur; it is invariably associated with an active vibratory movement quite different from the normal. This fact, in conjunction with those cited above, confirms the inference that a heightened contractile activity, by whatever means induced, is always associated with coagulative alterations in the contractile substance. If the movement can be made to retain its normal rhythm and character, even in media of widely different composition from the normal, no immediate coagulation occurs.

The appearance of rapid vibratory activity in a plate immersed in any solution is thus an indication that coagulative changes are in progress and will soon become visible. An observation that I have

[1] In solutions of sodium chloride containing any one of the following salts in certain determinate proportions the movement may preserve its normal character for considerable periods, varying from thirty minutes to two hours. The action depends on the cation of the added "antitoxic" salt, and the concentration required varies with the valence and decomposition-tension of the cation, as well as with certain other undetermined specific properties (thus the alkali earths have little or no action): — $MgCl_2$, $AgNO_3$, $MnSO_4$, $CdCl_2$, $ZnSO_4$, $Pb(NO_3)_2$, $UO_2(NO_3)_2$, $HgCl_2$, $Fe(NH_4)_2(SO_4)_2$, $AlCl_3$, $FeCl_3$, $Cr_2(SO_4)_3$, $CeCl_3$, $Di(NO_3)_3$, $SnCl_4$, $ZrCl_4$, $Th(NO_3)_4$. The action of salt solutions will be considered in detail in another paper.

frequently made on specimens of Eucharis kept for some days in standing dishes of sea water has a similar significance and is of interest here. In dying animals the swimming-plates coagulate and whiten, as in the above solutions; the change appears to be of the same essential nature as the coagulative process accompanying rigor mortis. Shortly before its appearance one often observes that those plates which are about to cease activity exhibit a continued and unintermittent beating movement of abnormally quick rhythm like that of the above vibratory movement, though never so rapid. The change in character of movement appears to be an indication that certain ante-mortem changes are in progress within the plate substance; these, as the later coagulation shows, consist partly in a progressive coagulation of the colloids of the contractile tissue. The more rapid rhythm of movement then observed is to be regarded as due, just as in the artificially accelerated movement described, to the presence of conditions — in this case deficient oxygenation or the presence of bacterial products — favoring coagulative changes in the fibrils, and hence accelerating the coagulative phase of the contraction process. This increased activity during the ante-mortem alterations bears a certain correspondence to the muscular twitching observed by Schiff and others during the early stages of rigor mortis.

Summary.

1. Artificially accelerated contractile activity in the swimming-plates of the Ctenophore Eucharis lobata is accompanied by visible coagulative alterations in the contractile fibrils.

2. During movement in the normal medium (sea water), and in artificial solutions in which movement retains its normal rhythm, no such coagulation occurs. Rapid vibratory movements induced by various solutions (*e.g.*, of weakly acidulated sodium salts) are invariably accompanied by rapid coagulation; this change is the more rapid the more energetic the contractile activity. In inactive plates it does not occur.

3. A connection between contractile activity and coagulative changes in the colloids of the contractile tissue is thus indicated. Presumably the contraction-phase of the beat is due to a coalescence (incipient coagulation) of colloid particles; this change is reversed during the relaxation-phase. During accelerated movement the reversal is incomplete, and the coagulation is therefore progressive.

LACTIC ACID IN INTERMEDIARY METABOLISM.

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Introduction.

JUST as dextrose is the sugar of the body, so para-lactic or d-lactic acid is the form of lactic acid found.

Moriya,[2] quoting the literature of the subject, says the proof that the lactic acid found in the body is the d-modification, was afforded by Wislicensus for muscle, by Gaglio for blood, and by Pautz for the vitreous humor. Moriya himself shows that d-lactic acid exists in the brain tissue of all animals, in lymph glands, kidney, thymus, spleen, pancreas, and suprarenal glands. The isolated zinc salt of the acid is in all cases a d-lactate. Morishima[3] proved that the d-lactate was present in normal liver, blood, muscle, kidney, and gastro-intestinal wall, and in the same organs, but in much larger quantities, in arsenical poisoning. Inactive or fermentation lactic acid was never found.

Morishima, however, noticed that if livers containing much glycogen were ground to a pulp and left to stand in the air at 16° to 18° for two or three days, the glycogen decreased and fermentation lactic acid was found in its stead. These conditions, however, were not those of normal life, putrefaction having always been present, with sulphuretted hydrogen distinctly perceptible in one of the cases. Such results are not convincing as regards what obtains in the living cells.

Marcuse[4] reports a large production of d-lactic acid in the muscles of frogs poisoned with strychnin, and a small excretion of this acid in

[1] Louis C. Tiffany Research Fellow in Physiology.

[2] Moriya: Zeitschrift für physiologische Chemie, 1905, xliii, p. 397.

[3] Morishima: Archiv für experimentelle Pathologie und Pharmakologie 1899, xliii, p. 217.

[4] Marcuse: Archiv für die gesammte Physiologie, 1886, xxxix, p. 425.

the urine. He believed the acid was largely destroyed in the liver, basing his opinion on the just reported discovery of Minkowski that the extirpation of the liver in geese leads to a large output of lactic acid in the urine. These experiments were followed by those of Werther[1] who compared the phenomena of tetanus and rigor mortis in frogs, noting that both were accompanied by a reduction of muscle glycogen and an increase in lactic acid.

Spiro[2] demonstrated that tetanus in dogs and rabbits resulted in a large increase in the amount of lactate present in the blood. Neither he nor Marcuse was able to detect lactic acid in the urine of men who danced or exercised hard in the gymnasium for three or four hours. However, Inouye and Saiki[3] have confirmed work by Araki[4] and have shown that d-lactic acid is eliminated in the urine of epileptics after the convulsive attack. Also Fuerth and Lockman[5] find d-lactic acid in the cerebro-spinal fluid of eclamptics, after the the seizure.

Araki[6] formulated the proposition that lactic acid (as well as sugar) appeared in the urine under conditions of insufficient oxygenation of the tissues, as may be brought about by carbon-monoxide, morphine, curarin, amyl nitrite, hydrocyanic acid, strychnin, and veratrin.

Zuntz[7] while living on top of Monte Rosa, observed that the atmosphere is sufficiently rarefied that if the respiration be partially impeded, as in the act of buttoning one's boots, a decided oppression results. During hard exercise at this height products of metabolism are formed which are not oxidized — products of an *anaerobic* cleavage. Although Zuntz[7] does not make the statement, it is to be expected that lactic acid was among these products. The formation of lactic acid, $C_3H_6O_3$, from the metabolism of dextrose, $C_6H_{12}O_6$, which accords with Hoppe-Seylers' conception of carbohydrate metabolism, is an excellent illustration of anaerobic cleavage.

Phosphorus and arsenical poisoning present conditions in which

[1] Werther: Archiv für die gesammte Physiologie, 1890, xlvi, p. 63.

[2] Spiro: Zeitschrift für physiologische Chemie, 1877, i, p. 111.

[3] Inouye and Saiki: Zeitschrift für physiologische Chemie, 1903, xxxvii, p. 205.

[4] Araki: Zeitschrift für physiologische Chemie, 1891, xv, p. 335.

[5] Fuerth and Lockman, Centralblatt für Gynaekologie, 1906, xxx, p. 41.

[6] Araki: Zeitschrift für physiologische Chemie, 1894, xix, p. 422.

[7] Durig and Zuntz: Archiv für Physiologie, 1904, Supplement Band, p. 416.

there is an accumulation of lactic acid in the blood and an elimination of it through the kidney.

Araki[1] finds the conditions similar in these two affections of the organism. He finds that anaemia is not the cause of the accumulation of lactic acid, but rather a reduced activity of the heart which slows the circulation and correspondingly diminishes the oxygen supply to the tissues.

Under normal conditions lactates burn readily in the organism when given either per os or subcutaneously.[2] When lactates burn, proteid metabolism is reduced, just as results when dextrose burns. Thus Weiske and Flechsig[3] found that 60 gm. of lactic acid (administered as a calcium salt) had the same sparing effect on the proteid metabolism in sheep as a like quantity of dextrose. When, however, lactates or dextrose normally produced in the body do not burn, but are eliminated in the urine, then proteid metabolism rises. This has long been known in phosphorus poisoning and in diabetes. Inouye and Saike quote cases of epileptic convulsions (lactic acid elimination) in which the urea excretion increased on an average of nine per cent on the day of the seizure.

One conception of the action of phosphorus is that it induces autolysis of the body's protoplasm (Jacoby,[4] Waldvogel[5]) since leucin, tyrosin and other amino acids may be eliminated in considerable quantity in the urine. Oswald[6] thinks that phosphorus destroys or weakens the anti-autolytic enzymes of the body. That autolytic enzymes do not have free control over the cells through the direct influence of phosphorus, is proved by the work of Ray, McDermott and Lusk.[7] These authors found that phosphorus injections raised the proteid metabolism of fasting dogs to 250, 260, 283, 248, 183 and 164 per cent of that found in inanition. They contrasted this increased proteid metabolism with that obtained in phlorhizin glycosuria, which is represented by increases to 540, 450, 340 and 340 per cent. When, however, they gave phlorhizin and obtained the increased metabolism and then injected phosphorus, this was not followed by any marked further increase in proteid metabolism.

[1] Araki: Zeitschrift für physiologische Chemie, 1893, xvii, p. 311.
[2] Araki: Zeitschrift für physiologische Chemie, 1894, xix, p. 455.
[3] Weiske and Flechsig: Journal für Landwirthschaft, 1889, xxxvii, p. 199.
[4] Jacoby: Zeitschrift für physiologische Chemie, 1900, xxx, p. 174.
[5] Waldvogel: Archiv für klinische Medizin, 1905, lxxxii, p. 437.
[6] Oswald: Biochemisches Centralblatt, 1905, iii, p. 365.
[7] Ray, McDermott and Lusk: This journal, 1899, iii, p 139.

Under these circumstances the glycosuria is the predominating influence removing dextrose as formed from proteid. The above named authors also reversed this experiment. They injected a dog with phosphorus and obtained a urine alkaline with ammonia and filled with tyrosin crystals. On administration of phlorhizin the urine became acid and the normal ratio between dextrose and nitrogen was readily obtained.[1]

From the character of these results it seemed very probable that the ammoniacal reaction of the urine in phosphorus poisoning was due to the formation of lactic acid in the organism, which had to be neutralized. Since the urine became acid once more on administration of phlorhizin, one might argue that the production of lactic acid ceased with the removal of dextrose from the body. It therefore might be inferred that lactic acid was a cleavage product of dextrose. If this were true, no lactic acid should be present in the blood and urine of a dog poisoned with phosphorus after phlorhizin diabetes is induced, which latter condition effects the removal of dextrose, the probable mother substance of lactic acid. The first part of the present research deals with this problem;[2] but before proceeding to its description, a few more words must be devoted to a consideration of the source of lactic acid in metabolism.

Minkowski[3] clearly showed that after extirpation of the liver in geese, ammonia and lactic acid replaced the customary uric acid in the excreta, and that this lactic acid was proportional to the amount of proteid destroyed and to the ammonia present in the urine. Ingestion of carbohydrates did not increase the quantity of lactic acid eliminated, and therefore Minkowski concluded that lactic acid owed its origin to proteid alone. Araki[4] subjects this to the criticism that the severity of the operation prevented the proper absorption of ingested carbohydrates.

Asher and Jackson,[5] by ligating the coeliac, superior and inferior mesenteric, phrenic and renal arteries and renal veins, cut off the blood supply to intestine, liver, spleen, pancreas, and kidneys of a

[1] The view advanced by RAY, MCDERMOTT and LUSK that the high proteid metabolism in phosphorus poisoning is due to the conversion of a part of the proteid molecule into fat, is scarcely tenable to-day.

[2] Preliminary communication by A. R. MANDEL: This journal, 1905, xiii, p. xvi.

[3] MINKOWSKI: *loc. cit.*

[4] ARAKI: Zeitschrift für physiologische Chemie, 1894, xix, p. 470.

[5] ASHER and JACKSON: Zeitschrift für Biologie, 1901, xli, p. 393.

dog. Circulation through the muscles and lungs was maintained by the beating heart. Under these circumstances lactic acid increased in the blood. Ingestion of carbohydrates, however, did not increase the lactic acid in the blood, and the authors conclude that the lactic acid is derived from proteid. While this latter is true, the experiments fail to show that carbohydrates may not normally be converted into lactic acid, for with the elimination of the pancreas from the circulation no ingested dextrose could have been destroyed.

A strict demonstration that lactic acid could have its origin from carbohydrates in metabolism came to our knowledge after the preliminary communication to the American Physiological Society had been presented from our laboratory, and after the completion of the greater part of the work included in this article. Embden,[1] in this still incompletely reported piece of work, showed that percolation with blood of a surviving liver rich in glycogen resulted in the appearance of lactic acid in the circulated fluid; also percolation of a glycogen-free liver with blood rich in sugar resulted in a very considerable formation of lactic acid, whereas a blood poor in sugar effected only a small production of the acid.

Stoklasa[2] has isolated an enzyme from muscle and other organs which has the power of converting dextrose into lactic acid. *It is therefore apparent that there is evidence that lactic acid may arise from both carbohydrate and from proteid metabolism.*

As regards the method of the origin of lactic acid from proteid metabolism two examples may be cited.

Neuberg and Langstein[3] found glycogen in the liver and lactic acid in the urine of a rabbit following the ingestion of alanin. The transformation of alanin, a cleavage product of proteid, into lactic acid may be written:

$$\underset{\text{Alanin}}{CH_3 \cdot CHNH_2 \cdot COOH} + H_2O = \underset{\text{Lactic acid.}}{CH_3 \cdot CHOH \cdot COOH} + NH_3$$

The second example is given by von Noorden and Embden,[4] who find that percolation of blood containing leucin through a surviving

[1] EMBDEN: VI internationaler Physiologischencongress, 1904: Centralblatt für Physiologie, 1905, xviii, p. 832.

[2] STOKLASA, JELINEK and CERNY: Centralblatt für Physiologie, 1902, xvi, p. 712.

[3] NEUBERG and LANGSTEIN: Archiv für Physiologie, Supplement Band, 1903, p. 514.

[4] VON NOORDEN and EMBDEN: Centralblatt für die gesammte Physiologie und Pathologie des Stoffwechsels, 1906, i. p. 2.

liver results in a production of acetone, which is found in the blood, and in a probable formation of lactic acid. This reaction would depend upon the cleavage of the leucin molecule, as is indicated in the following formula:

Radicle convertible into acetone — Alanin radicle

These authors find that blood containing alanin when percolated through a surviving liver loses its alanin, in the place of which lactic acid appears. One may therefore picture a considerable origin of lactic acid from proteid through simple hydrolytic cleavage and denitrogenization processes.

A final factor lies in the observation of Neuberg and Langstein, that alanin ingestion increases the glycogen in the liver, and this probably through a synthetic production from lactic acid formed. Such a process would indicate a possible course of the sugar production from proteid. This idea receives further confirmation from the experiments of Amalagia and Embden,[1] which have shown that the ingestion of d-alanin by a dog with pancreatic diabetes largely increases the sugar output in the urine.

Embden and Salomon[2] in two experiments have attempted to demonstrate the conversion of lactic acid into sugar. In one dog from which the pancreas had been removed a partial diabetes ensued. The urine became sugar-free; but on giving 13 gm. of sodium lactate 7.8 gm. of dextrose appeared in the urine. In another experiment with a diabetic dog, 35 gm. of sodium lactate were given through a stomach sound, and then (because of violent vomiting) 20 gm. were administered subcutaneously. The urine of the day before contained 9.7 gms. of sugar, and on the day of lactate ingestion 19.47 gm.

The results are open to the following criticisms: That the diabetes was not complete; that no record was made of the nitrogen elimination of the time; and also that the increased sugar elimination may have been due to the ingestion of sodium ions,[3] which Underhill[4] claims increases the permeability of the kidney epithelium for dextrose.

[1] Amalagia and Embden: Hofmeister's Beiträge, 1905, vii, p. 304.
[2] Embden and Salomon: Hofmeister's Beiträge, 1904, vi, p. 64.
[3] Fischer: University of California Publications, Physiology, 1903, i. p. 77.
[4] Underhill and Closson: This journal, 1905, xv, p 22.

This review of the literature enables an intelligible presentation of the following experiments.

Methods.

Nitrogen was estimated by the Kjeldahl method, ammonia by Folin's method, and dextrose by the method of Allihn. Lactic acid in the blood was estimated by the method described by Asher and Jackson.[1] Lactic acid in the urine was estimated as follows: 200 c.c. of urine were treated with 2,000 c.c. of 95 per cent alcohol. This was acidified with phosphoric acid and extracted with much ether. The ether extract was evaporated, taken up with water, filtered, digested with zinc carbonate, and filtered. The filtrate was treated with hydrogen sulphide, filtered and shaken with more ether. From this, through repetition of the treatment given the ether extract above, a solution of zinc lactate was obtained which yielded crystals on drying. The phlorhizin used was manufactured by Merck.

Dextrose is the Source of Lactic Acid in Phosphorus Poisoning.

Dog 1. The animal fasted three days (January 20–22) and then received phlorhizin in three gm. doses three times daily for nine days (January 23–31). During the last of five of these days (January 27–31) 2 c.c. of a 1 per cent solution of phosphorus oil was daily administered subcutaneously. The urine was only analyzed on alternate days, commencing January 26.

The results are presented in Table I.

This experiment demonstrates that lactic acid which is present in normal blood disappears from the blood when diabetes is induced and does not occur in the blood or urine even though phosphorus poisoning be induced. Duplicate analyses of 200 c.c. of blood were made in each case.

The experiment confirms the fact that nitrogen in the urine is not increased when phosphorus is administered after phlorhizin diabetes has been established, and that the D : N ratio is not affected by the phosphorus dosage.

Dog 2. The next experiment on a fasting dog indicates the usual effect of phosphorus injections on the fasting metabolism (see Table II).

The dog died during the night of November 14.

[1] Asher and Jackson: *Loc. cit.* p. 95.

TABLE I.

Dog I. Weight = 40 kg.

Date.	Urine.	Reaction.	Dextrose in urine.	Nitrogen in urine.	D : N	Zn lactate in urine.	Zn lactate in 100 c.c. blood.	Remarks.
1904	c.c.		grams	grams		grams	grams	
Jan. 22	....	acid	....	....	....	....	0.1054	Simple starvation.
" 23	....	....	....	....	....	....	....	Phlorhizin 3 times daily.
" 26	1320	acid	107.70	29.32	3.61	none	none	Phlorhizin.
" 27	....	....	....	....	....	....	....	Phlorhizin and phosphorus.
" 28	1800	acid	110.92	28.31	3.91[1]	....	....	" "
" 30	1500	acid	98.47	26.54	3.71	....	....	" "
" 31	1200	alkaline	87.17	24.16	3.61	none	none	Dog is very weak.

[1] The dog had severe convulsions twice on this day.

TABLE II.

Dog II. Weight = 16 Kg. Fasting since November 7th. Twelve hour periods.

Date.	Urine.	Reaction.	NH_3 in urine.	Nitrogen in urine.	Zn lactate acid from urine.	Remarks.
1903	c.c.		grams	grams	grams	
Nov. 9	90	acid	0.113	2.913	....	....
" 10	100	"	0.074	2.635	....	....
" 10	90	"	0.065	2.593	....	....
" 11	80	"	0.067	2.369	....	....
" 11	55	"	0.069	2.534	....	1 c.c. P* oil
" 12	110	"	0.077	2.854	....	....
" 12	75	"	0.073	2.627	....	3 c.c. P* oil
" 13	80	"	0.089	3.017	....	....
" 13	275	"	0.132	3.980	0.619	1 c.c. P* oil
" 14	500	Faintly acid	0.239	5.463	0.919	1 c.c. P* oil

* Phosphorus.

The dog's nitrogen metabolism rose from the starvation minimum of 2.369 gm. on November 11 to 5.463 gm. on November 14, an increase to 230 per cent. On the 14th 0.919 gm. of zinc lactate were recovered from the urine, an amount concomitant with a considerable accumulation of the acid in the tissues. The excretion of ammonia in the urine increased fourfold that of the fasting minimum.

An experiment like the last was now carried out, only after phosphorus poisoning was established, diabetes was induced with the result that lactic acid disappeared from both blood and urine. For the analysis of the last sample of blood 420 c.c. were drawn into two litres of alcohol:

TABLE III.

Dog III. Weight = 16.3 Kg. Fasting since November 17th. 12 hour periods.

Date.	Urine.	Reaction.	NH_3 in urine.	Nitrogen in urine.	Zn lactate from urine.	Zn lactate from 100 c.c. blood.	Remarks.
1903	c.c		grams	grams	grams	grams	
Nov. 21	50	acid	0.083	1.632	....	....	....
" 21	60	acid	0.112	2.065	....	....	1 c.c. P oil
" 22	50	acid	0.293	2.431	....	....	....
" 22	150	acid	0.313	2.784	....	....	1 c.c. P oil
" 23	100	acid	0.408	2.184	....	....	....
" 23	100	alk. + NH_3	0.631	3.138	2.060	0.158	....
" 24	325	alkaline	0.588	4.186	1.462	....	diabetes
" 24	?	acid	0.506	5.376	0.485	....	"
" 25	550	alkaline	0.644	6.048	none	....	"
" 25	400	acid	0.874	7.336	none	none	"

Through the influence of the phosphorus the nitrogen metabolism rose from 1.623 gm. on November 21st to 3.138 gm. on the 23d. Twelve hours' urine of this day showed the presence of 2.06 gm. of lactate, while the blood of the same period contained 0.158 per cent. On administering phlorhizin three times daily the excretion of lactic acid in the urine continued for twenty-four hours and then completely ceased. Lactic acid also disappeared from the blood. Either it might have burned in the organism, or as will be shown, it might have been converted into dextrose and eliminated in the urine. Ray,

McDermott, and Lusk[1] find that their Dog III, which, like the one just described, had been poisoned with phosphorus, eliminated 4.9 gm. of "extra sugar"[2] in his urine during the first twelve hours of phlorhizin administration. How much of this was derived from the body sugar and how much from lactic acid is, of course, undeterminable from the figures at hand.

The complete disappearance of lactic acid in phosphorus poisoning when diabetes is induced, indicates that lactic acid produced from the cleavage and denitrogenization of proteid, whether this occurs in the intestinal wall or in the liver or elsewhere, is first synthesized to dextrose probably within the liver, before further distribution to the tissues. In the case of phosphorus poisoning the distribution of dextrose takes place with resultant anaerobic cleavage leading to the production of lactic acid again. If diabetes be induced, this distribution of the sugar to the tissues is cut short by its complete elimination in the urine. Whether phosphorus poisoning completely or only partly destroys the capacity for oxidizing lactic acid, is not to be deduced from any experiments now at hand.

The cause of the appearance of leucin, tyrosin, and other amino acids in the urine and tissues in phosphorus poisoning may, in the light of the facts set forth, be more readily explained on the assumption that lactic acid partially inhibits the activity of the denitrogenizing enzymes, than by the theory of Oswald,[3] which declares that phosphorus *per se* destroys or weakens anti-autolytic enzymes.

Ingested *i*-lactates Reduce the Fasting Proteid Metabolism.

A dog which had fasted twenty-four hours was continued starving and on the fourth and fifth days 18.7 gm. of calcium lactate were administered per os. This corresponds to 11 gm. of lactic acid or 1 gm. per kilogram of dog. Diarrhœa ensued. The experiment may be tabulated as in Table IV.

The urine of May 13 was unfortunately lost. The experiment shows that the lactate given may reduce proteid metabolism. The absorption may have been incomplete on account of the diarrhœa. Whether dextrose in the same small quantity would have the same action on metabolism, is not as yet known.

[1] Ray, McDermott, and Lusk: *Loc. cit.* p. 146.

[2] Extra sugar is the amount of sugar eliminated in the urine which cannot be attributed to the proteid metabolism of the time.

[3] Oswald: *Loc. cit.*

It must be admitted that the above experiment is not of itself very convincing, for the reduction in the proteid metabolism is not large. Several other similar experiments were attempted, but in all either vomit or fæces became mixed with the urine, and administration of the lactate in this fashion was abandoned.

TABLE IV.

Dog. IV. Weight 11 Kg. Starvation.

Date.	Nitrogen in urine.	Remarks.
1905	grams	
May 8	2.90	....
" 9	2.82	....
" 10	2.76	18.7 g. Ca lactate
" 11	2.51	" " "
" 12	2.56	....

However, in the following section, it will be shown that this inactive form of lactic acid has the power of reducing proteid metabolism when administered in diabetes, and this reinforces our judgment that it may also do the same in health.

There is a Partial Conversion of Ingested Fermentation Lactic Acid into Dextrose.

In the first experiment 25 gm. of sodium lactate (Merck) were given to a diabetic dog. Dr. P. A. Levene kindly made a determination of the amount of carbon in this solution and found it to be 22.99 per cent. From this it may be calculated that the sodium lactate solution contained the equivalent of 57.5 per cent of lactic acid, a value adopted in our subsequent calculations. We found further the 0.5 gm. of the solution yielded 0.2984 gm. of lithium lactate, which indicates the equivalent of 54.18 per cent of lactic acid.

The 25 gm. of sodium lactate administered to the dog contained the equivalent of 14.37 gm. of lactic acid. It was given per os in 5 gm. doses and was considerably diluted with water. The dog was carefully watched and the urine was frequently collected through a

catheter. The lactate was retained by the dog and there was no diarrhœa. The following table represents the analytical data:

TABLE V.

Dog V. Weight = 12.2 Kg. Fasted since December 24th

Date.	Dextrose.	Nitrogen.	D : N	NH_3	Remarks.
1903	grams	grams			
Dec. 28	....	1.43	....	0.164	....
" 29	....	....	....	....	Diabetes
" 30 10 A.M.–4 P.M.	13.88	3.63	3.79	0.206	....
Dec. 30 4 P.M.–4 A.M.[1]	27.45	6.00	4.58	0.221	Lactate ingestion
Dec. 31 4 A.M.–12 M.	18.19	4.20	4.33	0.198	....
Dec. 31 12 M.–8 P.M.	18.82	4.67	4.03	....	....

[1] 5 c.c. sodium lactate at 4, 6:30, 9:30 P. M. and 12:30, 3 A. M.

The hourly nitrogen elimination in the different periods was as follows:

Dec. 1903.	Nitrogen per hour.	NH_3 per hour.
28 Fasting	0.120	0.014
30 Fasting and diabetic	0.605	0.034
30 Lactate ingestion	0.500	0.018
31 After-period I	0.525	0.025
31 After-period II	0.584	

The nitrogen elimination was increased to 500 per cent of that in starvation on the production of diabetes; it was diminished on administering fermentation lactic acid; and it returned nearly to its former level in a period beginning nine hours after the last dose of the lactate. The combustion of the lactic acid, therefore, reduced proteid metabolism. Combustion of the alkaline lactate apparently reduced the ammonia excretion of the period.

As regards the sugar output following the ingestion of the sodium lactate, it will be first necessary to recall that Stiles and Lusk[1] after a rigid critique of existing records regarding the D : N ratio in urine

[1] STILES and LUSK: This journal, 1903, x, p. 77.

of phlorhizin glycosuria in the fasting dogs, found it to be very constant at 3.65 gm. of dextrose to 1 of nitrogen. Now in Dog V, during the eleven hours of lactate ingestion and the following seventeen hours, 64.46 gm. of dextrose and 14.87 gm. of nitrogen were eliminated in the urine. But the sugar corresponding to this nitrogen metabolism should have been 14.87 (N) × 3.65 (D) or 54.27 gm. Since 64.46 gm. were actually present, it follows that 10.19 gm. of extra sugar were eliminated after the administration of 25 c.c. of concentrated syrup of sodium lactate containing 14.37 gm. of lactic acid. It may be calculated that 70 per cent of the lactic acid given was converted into dextrose while another portion burned and reduced the proteid metabolism.

A second experiment along similar lines was carried out on another dog, but in this case crystalline calcium lactate was given in such quantity as to represent approximately 1 gm. of lactic acid per kilogram of animal. The dog suffered from diarrhœa and we were therefore unable to obtain the night urine free from contamination. The collection of urine was, for this reason, confined to twelve hours after the ingestion of the lactate. The previous experiment, however, demonstrated that the greater part of the absorption of sodium lactate was complete nine hours after the last ingestion of the substance, and in another case (Dog VII) which will be cited the absorption of para-lactate was complete after nine hours.

The dog commenced his fast on March 26, at which time he weighed 12.8 kilograms. From March 27 he received 2 gm. of phlorhizin three times daily. The analytical data are as follows:

TABLE VI.

Dog VI. Twelve-hour periods.

Date.	Weight.	Dextrose in urine.	Nitrogen in urine.	D : N	Extra Dextrose.	l-lactic acid.
1904	kilos	grams	grams		grams	grams
March 30	11.2	20.92	5.53	3.78	....	....
" 31	10.9	20.56	5.13	4.00	1.84	2
April 1	10.5	16.56	3.63	4.56	3.31	11
" 2	....	15.23	3.63	4.20	1.98	11
" 3	....	17.85	3.70	4.80	4.35	10

Examination of this table will show (1) a considerable reduction of proteid metabolism following the ingestion of comparatively small quantities of inactive lactic acid and (2) the elimination of 3.31 gm. of "extra sugar" after giving 11 gm. of lactic acid to the dog and 4.35 gm. after giving 10 gm. Here then 30 and 43 per cent of the lactic acid has been converted into sugar by the organism.

That under favorable conditions half or more of the lactic acid ingested could be converted into dextrose, while the rest burned and reduced proteid metabolism, suggested the idea that it might be possible that the l- and d- components behaved differently in the diabetic organism, the latter being converted into dextrose, and the former burning in part, and in part being converted into dextrose.

There may be a Complete Conversion of *d*-lactic Acid into Dextrose.

The d-lactic acid used was obtained freshly prepared, from Kahlbaum. We are greatly indebted to the kindness of Dr. P. A. Levene who analyzed the substance and found that it contained carbon corresponding to a lactic acid content of 84.75 per cent while the acidity of solution represented 81.2 per cent of the acid. In the calculation of the following experiments use has been made of the first named value. Unfortunately, the para-lactic acid was not absolutely pure. Crystals of the zinc or lithium salts could not be directly obtained. On treating 1 c.c. with paraffin, according to the Asher-Jackson method employed for the analysis of blood, a zinc salt was obtained which weighed 0.7432 gm. (=0.48 gm. of lactic acid). This substance contained 11.40 per cent of water of crystallization and 27.94 per cent of ZnO. Both of these values are below those demanded for zinc para-lactate, but approximate the relative value in which water and ZnO are present in that salt. The substance therefore consists of para-lactate, with a certain amount of impurity.

In one experiment we injected 5 c.c. of the lactic acid solution (=4.26 gm. lactic acid) diluted with 100 c.c. of water and neutralized with sodium carbonate into the intestine of a phlorhizinized dog. The dog had previously been operated on, so that the upper part of the gut was adherent to the abdominal wall. The operation was performed by our colleague, Dr. George B. Wallace, to whom we desire to express our thanks.

The experimental results are as follows:

TABLE VII.

Dog. VII. Weight = 7 Kg. Starved since January 10. January 11-13, 1 gram phlorhizin every eight hours. On January 13, 1 gram phlorhizin every four hours, except midnight to morning.

Date.	Length of period.	Dextrose.	Nitrogen.	D : N	Extra Dextrose.	Nitrogen in urine in 3 hours.
1906 Jan. 13	hours 3	grams 5.064	grams 1.421	3.57	grams	grams 1.421
....	6[1]	10.400	2.786	3.74	1.99	1.393
....	3	6.248	1.389	4.50		1.389
....	11	18.061	5.051	3.57	....	1.377

[1] 4.26 grams of d-lactic acid as sodium salt into intestine direct.

This experiment shows (1) no reduction in proteid metabolism after ingesting d-lactic acid; such fall as occurs is the normal, even reduction noticed in fasting cases:[1] and it shows (2) the elimination of about 2 gm. of extra dextrose during nine hours following an ingestion 4.2 gm. of d-lactic acid. This method seemed to involve the possibility of a faulty absorption, and it therefore was decided to attempt subcutaneous injections of the d-lactate, as is set forth below.

The experiments were performed on two different dogs. After one day of fasting the dog was given, subcutaneously, every eight hours, 1 or 2 gm. of phlorhizin dissolved by warming in 15 to 30 c.c. of a one and two-tenths per cent sodium carbonate solution. On the days of the experiment phlorhizin was administered every four hours. In Dog IX a slight improvement in the usual procedure was adopted, and for the following reason. At the end of a long "night period" (sometimes for convenience nine or ten hours elapsed between injections) there may be a slight retention of dextrose in the organism,[2] which is thrown out in the urine by the morning dose of phlorhizin and this dextrose may slightly raise the ratio, as in the first morning urine of Dog. VIII. The improvement consisted in waiting an hour after the morning injection until this retained dextrose was swept out and then catheterizing and washing the bladder. The evenness of the D : N ratio may then be relied on.

[1] STILES and LUSK: *Loc. cit.*

[2] See STILES and LUSK: *Loc. cit.*

The effect of the subcutaneous injection of para-lactic acid (given as sodium lactate) in phlorhizin diabetes is presented in Table VIII.

Examination of Table VIII shows (1) that there is little or no reduction in the nitrogen elimination during the period of the subcutaneous injection of para-lactate; and (2) that para-lactic acid subcutaneously administered may be converted into dextrose to the extent of 57, 61 and 100 per cent of the substance given. Under certain circumstances, therefore, para-lactic acid may be completely converted into dextrose. It is possible that a variation in the time of absorption from the tissues was the cause of the variation in the quantity of dextrose produced after the injection of para-lactate.

On January 26, it is estimated that 3.76 gm. of extra dextrose were eliminated after the ingestion of 3.4 gm. of para-lactic acid. This estimate of "extra dextrose" is based on the quantity which could not have had its origin from the proteid metabolism of the time. If, however, one observes that 8.1 gm. of dextrose were excreted during the three-hour periods immediately preceding and following the nine-hour period of extra sugar elimination, one may compute an elimination of 24.3 gm. of dextrose for the nine-hour period. Since 27.4 gm. were actually eliminated, it follows that 3.1 gm. were extra sugar. This result, however, is only obtained because the nitrogen elimination is stationary. A similar calculation on January 27 would have shown no "extra dextrose" whatever. It is, therefore, absolutely essential to follow the nitrogen excretion in all well conducted experiments of this sort.

Investigators who desire to determine the sugar production in the organism as caused by any given substance, will find this method easier and of greater reliability than that in which de-pancreatized dogs are employed (compare with critique on p. 134).

Stiles and Lusk[1] showed that dextrose injected subcutaneously into phlorhizinized dogs is completely eliminated in the urine within six to nine hours. It is evident from the work now presented that d-lactic acid may be absorbed from the subcutaneous tissue and carried by the blood to the organs where a sugar synthesis is effected (liver, muscles?) and there be converted into dextrose.

Claude Bernard showed that dextrose, whether derived from proteid or starch, was convertible into glycogen, and this again was changeable back into dextrose. The present research adds lactic acid to

[1] STILES and LUSK: *Loc. cit.*

TABLE VIII.

Dogs VIII and IX.

Dog.	Date.	Weight.	Length of period.	Amount of urine.	Dextrose.	Nitrogen.	D : N	Extra D.	d-lactic acid subcutaneous.	Nitrogen in urine in 3 hours.	Remarks.
	1906	kilos	hours	c.c.	grams	grams		grams	grams	grams	
VIII	Jan. 2	12.2	3	65	6.840	1.792	3.82	....	....	1.79	This dog fasted from December 29th and was diabetic from December 30th.
			3	88	6.816	1.869	3.64	....	4.26	1.87	
			6	174	15.848	3.920	$\frac{8}{21}$ (3.65)	1.54	....	1.96	
			3	80	8.019	1.904	(3.65)	1.07	....	1.90	
			?	....	14.096	3.850	3.66	(=61%)			
IX	Jan. 26	15.6	3	110	8.100	2.268	3.57	....	....	2.27	This dog fasted from January 23d and was diabetic from January 24th.
			9	380	27.408	6.624	(3.57)	3.76	3.4	2.21	
			3	....	8.103	2.317	3.50	(=110%)	....	2.32	
	Jan. 27		?	....	4.602	1.940	3.56				
			9	490	24.465	6.718	(3.56)	2.44	4.26	2.25	
			3	130	8.800	2.487	3.54	(=57%)	....	2.49	

In this table bracketed figures in the D : N column represent the assumed D : N ratio during the period of elimination of extra dextrose derived from lactic acid. This assumed ratio is based upon that actually found in the simple fasting periods.

both ends of this chain, in showing the following possible progression of events: lactic acid, dextrose, glycogen, dextrose, lactic acid.

Summary.

1. Lactic acid disappears from the blood and urine in phosphorus poisoning when phlorhizin glycosuria is induced. This indicates that lactic acid produced from the cleavage and denitrogenization of proteid, whether this occurs in the intestinal wall or in the liver or elsewhere, is first synthesized to dextrose within the organism (liver) before further distribution to the tissues. In the case of simple phosphorus poisoning this distribution of dextrose takes place with resultant anaerobic cleavage leading to a second production of lactic acid.

When diabetes is present, or when for any reason dextrose is converted only as far as the lactic acid stage, or when the mammary glands utilize dextrose to form milk-sugar, then the cells affected become "sugar-hungry cells," which attract fat in greater quantity than they can burn it (fatty infiltration). This picture of what is common to these conditions represents the standpoint developed in previous papers from this laboratory.

2. There is a partial conversion of i-lactic acid into dextrose in the diabetic organism. It appears that one-half and more of the lactic acid is so transformed while the rest burns and reduces proteid metabolism.

3. There may be a complete conversion of d-lactic acid into dextrose in the diabetic organism. There is never any considerable reduction of proteid metabolism indicative of a general combustion of a d-lactic acid when this is administered to a phlorhizinized dog.

THE PATHS OF EXCRETION FOR INORGANIC COMPOUNDS. II.—THE EXCRETION OF BARIUM.

By LAFAYETTE B. MENDEL and DUDLEY F. SICHER.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

I.

THE considerations which led to the series of observations planned to extend our knowledge of the paths of excretion of various substances have been pointed out in the earlier paper.[1] The study of the elimination of strontium there recorded showed that strontium salts are excreted to a relatively small extent only by the kidneys, even after direct introduction into the circulation. The elimination in the urine begins soon, and ceases usually within twenty-four hours. The larger portion of the strontium excreted is found in the fæces, whether the introduction of the element be per os, or parenterally, *i. e.* subcutaneously, intraperitoneally or intravenously. The rate of elimination is slow, and the place is apparently restricted to the region of the alimentary tract beyond the stomach.

The present experiments were attempted in order to ascertain whether barium salts, like those of the chemically related elements calcium, magnesium, and strontium, are likewise excreted by the bowel. To this question the literature regarding the pharmacology and toxicology of barium compounds gives no decisive answer.[2] The earlier investigators even differed as to the possibility of barium elimination by the kidneys in the few cases where the question was experimentally studied. J. Neumann,[3] however, definitely showed that after administration of barium chloride in man, dogs, and rabbits,

[1] Cf. MENDEL and THACHER: On the excretion of strontium, This journal, 1904, xi, p. 5.

[2] Cf. RICHET: Dictionnaire de physiologie; Article, Baryum; HEFFTER: Ergebnisse der Physiologie, 1903, ii, I, p. 121; KUNKEL: Handbuch der Toxicologie, p. 112.

[3] J. NEUMANN: Archiv für die gesammte Physiologie, 1885, xxxvi, p. 576.

barium reappears in the urine. No quantitative data are given regarding the extent of the elimination. In the dog the element was always found in the urine after administration of 0.1 gm. of barium chloride. After ingestion of the same amount in man, the barium was not detected in the urine, at least within the next few hours. With an intake of 0.3 gm. the urine contained barium within eight hours, and the administration of 0.5 gm. of barium chloride was followed by a detectable excretion of the alkali-earth metal within two hours. Santi[1] detected barium in the blood and urine of a dog poisoned with barium chloride. The failure of barium to be deposited as insoluble sulphate in the presence of soluble sulphates in the body is doubtless attributable to the simultaneous presence of other compounds, especially alkali bicarbonate and carbonic acid, which interfere with the reaction indicated. Bary[2] is said to have detected barium in the urine after subcutaneous administration of its salts.

Regarding other paths of elimination authentic statements are wanting. Neumann alone mentions the detection of barium in the saliva of man after ingestion of 0.5–0.8 gm. of barium chloride; and Lewin[3] states that he could detect barium spectroscopically in the stomach wall after subcutaneous administration of poisonous doses to animals. In the textbooks of Kobert, Kunkel and Cushny the probable excretory function of the bowel for barium salts is suggested, undoubtedly in consideration of the analogous behavior of the closely related element calcium. The question can be settled definitely only by the parenteral administration of the element, intravenously, subcutaneously, or intraperitoneally; since otherwise the presence of barium in the fæces may be attributable to failure of absorption as well as to excretion into the bowel.

II.

In our experiments barium chloride was administered to dogs subcutaneously in sterile solutions containing 5 mgm. $BaCl_2 . 2\ H_2O$ (2.8 mgm. Ba) per c.c.[4] The injections were usually scattered to

[1] SANTI: Gazzetta chimica italiana, 1903, xxxiii, II, p. 202.

[2] BARY: Dissertation, Dorpat, 1888. The original paper was not accessible to us.

[3] LEWIN: Deutsche medizinische Wochenschrift, 1906, p. 173.

[4] In determining the toxic dose of barium chloride we have noted a marked discrepancy between v. JAKSCH: Die Vergiftungen, p. 79, and other writers on

facilitate rapid absorption, and were made during slight ether anæsthesia. To counteract as far as possible the well-known diarrhœal effects of the salt, paper pulp was fed with the food. The fæces were thereby rendered more firm and less liable to contaminate the urine collected in the cages. Bone meal, which usually serves so well to give solid texture to the stools, was avoided in this case in order to eliminate every excess of calcium from the materials to be analyzed. Bread and meat, both relatively deficient in calcium salts, formed the diet.

Methods of analysis. — The dried materials (fæces, urine) were incinerated with ammonium nitrate in a porcelain crucible and then fused in a nickel crucible with sodium carbonate and potassium hydrate. The fusion mass (containing barium as insoluble carbonate) was extracted with hot water, the insoluble carbonates filtered off and digested with dilute hydrochloric acid, the solution of chlorides then being evaporated to dryness. The residue was now dissolved in an excess of hydrochloric acid and diluted to a large volume (300–400 c.c.), whereupon a little sulphuric acid was added and the insoluble sulphate allowed to settle in the usual manner. Where possible it was weighed and always examined spectroscopically for barium. Under the conditions noted, calcium did not interfere with the results; and with this procedure 3 mgm. of barium added to the quantities of urine usually employed could be detected.

Illustrative Protocols.

I. **Is barium eliminated with the fæces?** — A bitch weighing 11 kilos received 56 mgm. of barium. A fresh diarrhœal stool collected about 20 hours afterwards gave the test for barium. On the second day fæces collected directly without possible contamination with urine also gave a fairly strong test.

II. **How long does the elimination of barium by the kidneys and intestine continue?** — The bitch of Experiment I, after some days, received an injection of 98 mgm. of barium. As usual there was marked local irritation. Uncontaminated urine (275 c.c.) collected after 18 hours gave a strong test for barium. Diarrhœal stools gave a fair test. Urine

toxicology. v. Jaksch gives the lethal dose of barium chloride for man as 0.2 gm., whereas others ranged from 2 to 15 gm. The medicinal dose is stated as 0.03–0.13 gm. Neumann himself took as much as 0.8 gm. at once, and our animals received more than 0.2 gm. subcutaneously at one dose without fatal results. The statement of v. Jaksch is doubtless incorrect.

(350 c.c.) collected on the second day gave no test for barium. The freshly collected fæces of the third and fourth days gave a strong test for barium. These and further details are summarized below.

Period after injection.	Tests for barium in Urine.	Fæces.
18 hours.	Strong.	Faint.
2 days.	Negative.	Strong.
3 "	"	
4 "	....	Medium.
5 "	....	Faint.
8 "	....	Negative.

III. **Barium in the intestinal contents.** — After three weeks the same bitch received 242 mgm. of barium subcutaneously. The animal showed symptoms of severe intoxication and died in two hours. The mixed urine and fæces voided before death gave distinct tests for barium, indicating the early beginning of the excretion of the salt. The intestine did not yet present any evidence of marked changes at the autopsy. The removed contents of the entire intestine gave a faint test for barium.

IV. **Quantitative data on barium elimination.** — A dog weighing 12½ kilos received 134 mgm. of barium subcutaneously. In 100 c.c. of urine collected on the following morning no barium could be detected. A later portion presumably containing some barium was lost. Urine collected on the 4–6 days contained no detectable traces of the element. There was, notably, an absence of diarrhœa in the first days of this experiment, and with the concentrated diet of meat no fæces were obtained until the sixth day. They contained 7.8 mgms. of barium. On the eleventh day fæces containing 7.7 mgms. of barium were again obtained. Fæces collected at varying intervals after this also gave distinct evidence of barium. The data are here summarized:

Period after injection.	Barium tests in the Urine.	Fæces.
18 hours.	Negative.	....
1 day.	Lost.	....
4 days.	Negative.	
6 "		7.8 mgm.
11 "	....	7.7 "
12–13 days.	....	Distinct.
20 days.	....	Distinct.

III.

In an earlier paper the writer[1] ventured the suggestion of a functional connection between the physiological action of certain salts on

[1] MENDEL and THACHER: This journal, 1904, xi, pp. 15–16.

the gut and their passage through the walls of the intestinal tract. For the alkali-earths this path of elimination has now been ascertained. Magnus[1] and Kress[2] have since demonstrated that barium chloride acts as a local stimulant to the isolated intestinal musculature, even in the ganglion-free preparations after the removal of the plexus of Auerbach. In discussing the mode of action of the saline purgatives J. B. McCallum writes: "Sie wirken nicht desshalb, weil sie etwa nach Einspritzung unter die Haut oder in's Blut in den Darm abgesondert werden, eine Ansicht die vor kurzem von Mendel und Thacher ausgesprochen worden ist. Die Ansicht dieser Autoren wird dadurch widerlegt, dass die salinischen Lösungen viel schneller wirken, wenn sie in's Blut eingespritzt oder auf die peritoneale Oberfläche des Darmes gebracht werden, als wenn sie in das Lumen des Darmes eingebracht werden."[3] We have not intended to imply that the barium salts must enter the lumen of the intestine before they can unfold their characteristic peristaltic reaction. The facts merely support the idea of an intimate relation between stimulation of a neuro-muscular apparatus and glandular excretion in the same (complex) organ.

IV.

Summary.

There is a close resemblance between the salts of barium and strontium in their mode of excretion from the animal body. Barium introduced subcutaneously in the form of the soluble chloride in non-lethal doses is eliminated to a very small extent only by the kidneys, a detectable excretion through this channel ceasing within a few hours.

Barium is eliminated to a larger extent with the fæces, being conveyed from the circulation into the alimentary tract.[4] The actual quantities leaving the body by this path at one time are small; the process may continue for a long period and considerable amounts of barium may be deposited in the organism.

[1] Magnus: Archiv fur die gesammte Physiologie, 1905, cviii, p. 1.

[2] Kress: *Ibid.*, 1905, cix, p. 11.

[3] J. B. MacCallum: *Ibid.*, 1904, civ, p. 427.

[4] The elimination of radium takes place in a similar way according to observations published by Berg and Welker: Journal of biological chemistry, 1906, i p. 371, since the above was printed.

THE PATHS OF EXCRETION FOR INORGANIC COMPOUNDS. III.—THE EXCRETION OF RUBIDIUM.

By LAFAYETTE B. MENDEL and OLIVER E. CLOSSON.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

I.

THE observations of Good[1] on lithium and the experimental studies in this laboratory by Hanford[2] on cæsium indicate a noteworthy difference in the mode of elimination of these alkali metals in contrast with corresponding salts of the alkali earths.[3] The latter have been shown to leave the body in far greater quantity by the bowel than in the urine, the excretion through the kidney lasting during a brief period only, whereas the fæces may continue to carry away the foreign element for many days. Much information is still necessary before the variable rate and paths of elimination of inorganic compounds can be adequately interpreted. For example, potassium bromide injected into the body may reappear in a few minutes in the excretions, the output reaching its maximum in a few hours and then continuing at a lower level for a few weeks.[4] Again, thorium is neither absorbed nor excreted through the intestine, being eliminated by the kidneys alone.[5] Problems regarding the retention and deposition of foreign elements likewise arise in this connection.

The plan of the present experiments on the excretion of rubidium was essentially like that followed in the earlier studies of this series. The element was always introduced parenterally so that its elimina-

[1] Good: American journal of the medical sciences, 1903, cxxv, p. 273.

[2] Hanford: This journal, 1903, ix, p. 214.

[3] Mendel and Thacher: This journal, 1904, xi, p. 5; Mendel and Sicher: This journal, 1906, xvi, p. 147.

[4] Cf. Lewin: Deutsche medizinische Wochenschrift, 1906, p. 169.

[5] Brown and Sollmann: Proceedings of the society for experimental biology and medicine, 1905, ii, p. 55.

tion by the bowel could be studied without the possible error arising from unabsorbed rubidium ingested per os. We are not aware of any other experimental investigation on the subject.

II.

Methods. — Rubidium chloride was the salt used in all of the trials, the mode of introduction into the body being either subcutaneous or intravenous. The animals were kept in carefully cleaned metal-lined metabolism cages, the urine and fæces being collected separately. An attempt was made to estimate the quantities of rubidium eliminated or stored in various tissues, by applying the spectroscopic method of Gooch and Phinney.[1] By means of a hollow coil of platinum wire holding a definite quantity of liquid it is possible to bring definite amounts of any soluble substance into a flame to be viewed spectroscopically. A comparison of the brightness of two spectra produced in close succession can be made with reasonable accuracy; and by gradual dilutions and successive testings a solution of rubidium of unknown strength can be brought to the point of containing as much rubidium to the coil-full as a standard solution of known strength. By noting the final volume to which such an unknown solution is brought in the process of equalizing its spectrum with that of the standard its content of rubidium can be determined.

Among various disturbing factors the influence of the presence of other salts, notably those of potassium and sodium is the most serious, since the relative brilliance of the spectra is thereby considerably altered. This was in part overcome, however, by introducing into the standard rubidium solutions used for comparison, quantities of the other salts somewhat comparable with those occurring in the undetermined fluids to be examined. The error from this source nevertheless remains as an uncertain element. The coils used in our experiments were made of thirty turns of No. 28 platinum wire (0.32 mm. in diameter), forming a spiral 10 mm. long and 2 mm. in diameter and twisted together at the ends to form a long handle. These coils, holding $\frac{1}{20}$ c.c. of fluid were manipulated according to the directions of Gooch and Phinney, and were dried before being introduced into the flame. The method is far from being an accurate one, the errors sometimes reaching twenty per cent, even under more favorable conditions. Our quantitative results are at most roughly approximate;

[1] Gooch and Phinney: American journal of science, 1892, xliv, p. 392.

but in view of the difficulties attending the estimation of rubidium present in complex fluids in amounts aggregating a few milligrams only, they are presented for the comparative values which they fairly afford.

When sufficient rubidium was present in the urine, the element was estimated directly by comparison with the standards of series A below. The usual procedure for urine, fæces, and tissues consisted in evaporating to dryness with nitric acid, adding sulphuric acid and extracting the charred masses thoroughly with water. The extracts were concentrated and filtered and the process was repeated, if necessary, to remove more organic matter. The comparisons were then made with the standards of series B or C below.

The standard solutions for comparison of the flame spectra were prepared as follows:

A. Dog's urine diluted with an equal volume of water and divided into samples containing the number of milligrams of rubidium chloride indicated below.

(1) 1.0 (2) 0.5 (3) 0.33 (4) 0.25 (5) 0.166.

B. Solutions of rubidium sulphate (calculated as chloride) containing the inorganic urinary constituents in about twice their normal concentration. RbCl in milligrams per c.c.:

(1) 0.33 (2) 0.25 (3) 0.166.

C. Solutions like those of series B, the urine salts being present in about four times their normal concentration.

The results are all expressed in terms of rubidium chloride. The experimental work was undertaken by Mr. Closson.

Illustrative Protocols.

I. **The paths of elimination.** — A rabbit weighing 1.75 kilos received subcutaneously 0.5 gm. of rubidium chloride (285 mgm. per kilo) dissolved in 25 c.c. of water. The animal died on the 19th day thereafter, presumably of an infection then prevalent. The figures recorded for the quantities of rubidium recovered in the urine and fæces are doubtless too high owing to early inexperience with the method of estimation. The characteristic features are clearly indicated: the kidneys form the chief organ of excretion, and the elimination is gradual, being prolonged over many days.[1]

[1] Care was taken to avoid any error arising from continued contamination of he cages used.

Day	Rubidium chloride in Urine. mgm.	Fæces. mgm.
1	125	7
2	160	3
3		
4	60	
5	50	
6	15	trace
7	15	
8		
9		
10		
11	20	?
12		
13		
14		
15		
16	5	none
17		
18	trace	
19	none	

II. **Prolonged elimination in the urine.** — A dog weighing 7.5 kilos received subcutaneously 457 mgm. of rubidium chloride (63 mgm. per kilo) in 50 c.c. of water. After 13 days of observation the animal was kept in the stalls for a time, and on the 29th day again returned to a cage never previously used in experiments with rubidium. The fæces collected during the first four days contained no detectable traces of rubidium. The elimination (RbCl) in the urine of successive days was: (1) 25 mgm., (2) 3.5 mgm., (3–7) 9 mgm., averaging 2.25 mgm. per day, (8–13) average. 0.33 mgm. per day, (29–34) 2 mgm., averaging 0.3 mgm. per day.

III. **Retention and immediate distribution of rubidium in the body.** — 1. A cat weighing 3.1 kilos received, during anæsthesia, a subcutaneous injection of 625 mgm. of rubidium chloride (200 mgm. per kilo) dissolved in 25 c.c. of water. The animal drank water very freely as is characteristic in certain forms of gastro-intestinal irritation. The excretion of rubidium during the next six days was estimated. (See table on next page.)

2. The cat, now weighing 2.75 kilos, again received 670 mgm. of rubidium chloride (244 mgm. per kilo) subcutaneously in 27 c.c. of fluid. The animal showed a good appetite and considerable thirst until the twelfth day when it refused food. The following day the presence of bloody vomit in the cage suggested gastro-intestinal irritation. The successive daily outputs of RbCl in the urine of the 14 days of this period were as follows, expressed in milligrams: 100, 20, 16, 13, 6, 7, 1.5, (1.5, 1.5, 1.5), ?, (2, 2, 2,), a total of 175 mgm. The fæces during

this period contained traces from day to day, the soft stools of the first day including $1\frac{1}{4}$ mgm. with a total of 2.5 mgm. in 14 days. Thus in all 178 mgm. were recovered.

Experiment III, 1, page 155.

Day.	Rubidium chloride in		Character of the fæces.
	Urine.	Fæces.	
	mgm.	mgm.	
1 (18 hours)	8	No fæces	
2	13	Trace	24 gm., well formed.
3	3	17	46 " soft.
4	6	None	6 "
5	3	1.5	29 " soft.
6 (7 hours)	7	No fæces	
Injected, 625 mgm.; recovered 58.5 mgm. RbCl.			

3. The cat, now weighing 1.9 kilos next received an intravenous injection of 300 mgm. of rubidium chloride in 45 c.c. of water, with aseptic precautions, during anæsthesia. The infusion into a jugular vein lasted one hour. The animal did not rally well and showed an excessive thirst. The following day the cat was bled to death and portions of the body were analyzed.

Distribution of Rubidium in the Body.

Part analyzed.	Quantity used.	Rb (as RbCl) recovered.	RbCl per gm. of substance.
	gm.	mgm.	mgm.
Muscle of hind limb	50	50.0	1.00
Liver and gall bladder	90	40.0	0.44
Brain and cord	28	5.00	0.18
Blood	50	5.00	0.10
Contents of small intestine . . .	4	1.00	0.25
Ditto, large intestine and fæces .	16	0.35	0.02
Urine (24 hours)	40	17.00	0.43

IV. **Distribution of residual rubidium after prolonged excretion.** — A cat weighing 4.7 kilos received an intravenous injection of 598 mgm. of rubidium chloride dissolved in 60 c.c. of water during a period of fifty minutes.[1] The animal recovered readily. Twelve c.c. of saliva called forth by the ether used during the operation contained no rubidium. In a subsequent prolonged inhalation of ether 30 c.c. of saliva were collected, giving a detectable trace of the element. Urine and fæces were collected during the following eighteen days. In the fæces, well formed during the entire period, no rubidium was found; the urine contained quantities ranging from 3 to 15 mgm. (RbCl) per day, a total of over 115 mgm. being recovered. An attempt to induce diarrhœa by subcutaneous injection of barium chloride, with the purpose of noting whether rubidium would make its appearance in the fæces under such conditions, proved fatal. Since three-quarters of the rubidium injected still remained unaccounted for, various parts of the body were subjected to quantitative analysis for rubidium with the results noted in the table on page 158.

V. **Conditions modifying the elimination of rubidium.** — In Experiment II no rubidium was detected in the fæces after subcutaneous injection of 63 mgm. of RbCl per kilo. The animal was fed bones with the food, and the fæces were always hard and dry, indicating marked absorption of water in the large intestine. The following trial shows that when the intestinal contents are hurried forward more rapidly rubidium may be eliminated by the bowel also, in the early periods. Five weeks after the first injection the dog received less than 40 mgm. RbCl per kilo slowly injected into the jugular vein during anæsthesia. The urine of the subsequent day contained 4.5 mgm. of rubidium, the daily output then gradually decreasing to mere traces after the tenth day. In the fæces of the first day (8 gm.) no rubidium was noted; on the second day 24 gm. contained 0.25 mgm. of RbCl. The abundant fæces of the next three days contained traces, after which none could be detected in the stools.

III.

The observations just recorded disclose many points of resemblance in the behavior of rubidium salts and those of lithium and cæsium. All of them may be excreted by way of the intestine as well as through the kidneys. By far the greater quantity, however, appears to leave the body in the urine, in marked contrast with the conditions prevailing after the introduction of strontium or

[1] The action of rubidium chloride introduced directly into the circulation has been studied by Botkin, Jr.: Centralblatt für die medizinische Wissenschaften, 1885, xxiii, p. 849.

barium chloride, as shown in the earlier papers in this series. The process of elimination may be prolonged over many days. Good noted the reappearance of injected lithium salt in ten minutes and

Part analyzed.	Quantity used.	RbCl found.	RbCl per gm. of substance.
	gm.	mgm.	mgm.
Contents of stomach . .	10	1.0	0.10
Ditto, small intestine . .	15	3.0	0.20
Stomach	20	1.2	0.06
Small intestine	54	2.5	0.05
Large intestine	13	0.4	0.03
Contents of gall bladder .	5	0.1	0.02
Liver	130	3.5	0.03
Spleen	18	0.5	0.03
Omentum	150	Trace	....
Subcutaneous fat . . .	50	?	....
Kidneys	50	4.0	0.08
Muscle, heart	23	2.5	0.10
Muscle, hind leg	90	9.0	0.10
Muscle, lumbar region . .	80	9.5	0.12
Bloody pleural exudate .	30	3.5	0.12
Lungs (bloody)	45	8.0	0.17
Brain	28	0.8	0.03
Spinal cord	10	0.5	0.05
Fæces	31	None	....

a continued excretion for twenty-three days after the same single dose. Hanford found that cæsium elimination persisted many days after the last feeding. The protracted excretion of rubidium was noted in all of our experiments, and is evident in protocol II, for example, in which the element was still detected thirty-four days after a single injection.

In considering the readiness with which rubidium was eliminated with the fæces in different experiments we have gained the impression that the loss by the bowel was greater in those cases where

there was some evidence of a tendency to diarrhœa and the stools were less well formed. Good has observed that the introduction of lithium chloride is followed by gastro-enteritis connected, as he believes, with the excretion of the metal through the bowel wall. This action is caused by many other substances (Sr, Ba, Hg, As,) which are eliminated in the same manner. A glance at the distribution of rubidium along the alimentary tract (*e. g.* in Experiment IV) shows that the contents of the stomach and small intestine may contain considerable quantities of the element which is apparently reabsorbed in the large intestine and thus fails to make its appearance finally in the fæces. When conditions favor the more rapid transport of the materials through the gut and absorption is less complete (as evidenced by the soft stools) more rubidium is excreted by this channel. (*Cf.* Experiment III, 1.)

Where is the rubidium transferred into the gastro-intestinal canal? That it may be secreted in the saliva was noted in Experiment IV. This corresponds with the behavior of many halogen salts of the alkali metals, notably lithium and cæsium; whereas not all salts reappear in this way, the ferrocyanides forming one of the exceptions.[1] It is not unlikely, according to observations of Bonanni,[2] that the stomach wall also is selective in its secretion activity towards foreign salts. In dogs with a Heidenhain-Pawlow isolated fundus-sac he noted that after introduction of a considerable variety of compounds, including strontium, into the stomach proper, only NaI, NaBr, LiCl, and morphine were secreted into the sequestered portion of the organ. Rubidium has been found by us in the walls of the entire small intestine and stomach. Its presence in the contents of the gall bladder indicates an elimination in the bile also, as happens with copper, lead, zinc, iodine, and the ferrocyanides.

Most of the tissues participate in the temporary deposition or retention of rubidium, but the muscles form the chief storehouse. In this they surpass the liver as will be seen in Experiment III, where the analysis was made soon after the injection, as well as in Experiment IV, in which the animal was killed eighteen days after the last introduction of the salt. Comparable conditions were found in Good's experiments with lithium, the physiological action of which rubidium seems to resemble in many respects.

[1] For the references to the literature on this point see HANFORD: *Loc. cit.* p. 236; also LEWIN: *Loc. cit.*

[2] BONANNI: Abstract in Zentralblatt für Physiologie, 1905, xix. p. 152.

FURTHER STUDIES ON THE PHYSIOLOGY OF HEART BLOCK.—THE EFFECTS OF EXTRA SYSTOLES UPON THE DOG'S HEART AND UPON STRIPS OF TERRAPIN'S VENTRICLE IN THE VARIOUS STAGES OF BLOCK.[1]

By JOSEPH ERLANGER.

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Introduction.

IN a paper published in 1905 and entitled "Nachweis der Automatie der (mit den Vorhöfen oder Vorhofresten in Verbindung stehenden) Kammern bzw. Verbindungsfasern des Säugethierherzens durch Auslösung ventriculärer Extrasystolen" Hering[2] has shown that when the ventricles of the mammalian heart are beating automatically, or rather, independently, of the auricles, induced ventricular extra systoles are not followed by compensatory pauses. According to Hering the induced extra systoles either have the same duration as, or they may be slightly longer than, the spontaneous ventricular cycles. This observation, when slightly modified to suit the conditions which, according to Hering, obtain in the whole ventricle, agrees with the observation of Woodworth.[3] The latter investigator studied the properties of the isolated apex of the dog's heart kept beating by the method of Porter. He found that "the extra contraction is followed by a shortened but variable pause."

Somewhat later, Hering had the opportunity of confirming his above-mentioned observation after cutting the auriculo-ventricular bundle of His in the mammalian heart. In this later publication[4] Hering says,[5] "The method recently used by me to demonstrate the

[1] An abstract of this paper was read before the Society for experimental Biology and Medicine, on Feb. 21, 1906.

[2] Hering: Archiv für die gesammte Physiologie, 1905, cvii, p. 108.

[3] Woodworth: This journal, 1903, viii, p. 213.

[4] Hering: Archiv für die gesammte Physiologie, 1905, cviii, p. 276.

[5] "Die kürzlich von mir angewandete Methode die Automatie der Ventrikeln durch Auslösung ventrikulären Extrasystolen nachzuweisen kam mir bei diesen

automatism of the ventricles by inducing ventricular extra systoles was of great service in these experiments. . . . The ventricular extra period may be shorter or longer than a normal period, but it is never compensatory."

Finally, referring to my experiments on heart block Hering says,[1] "His results upon completely clamping the bundle of His in dogs agree perfectly with the results of my experiments. His experiments lack only the evidence that the ventricles beat automatically and that the conduction of the excitation no longer takes place."[2] Since Hering in this connection has studied the heart in complete block only, he doubtless here refers to those parts of my experiments which deal with complete heart block; he certainly does not mean to include in this criticism those parts of my experiments in which the block was presumably partial. Neither does Hering state specifically in his brief criticism of my experiments, the method that should have been used to demonstrate the automaticity of the ventricles when the block was presumably complete. However, we may be justified in inferring from what has gone before that the method of induced extra systoles should have been employed. It is therefore implied that, in order to prove that the ventricles are beating independently of the auricles, it suffices to show that induced extra systoles of the ventricles have approximately the same duration as the spontaneous ventricular cycles. It should, however, again be added that Hering has performed no experiments to determine whether or not his method will serve to distinguish complete from partial heart block.

The method which was used in my published experiments[3] to determine the existing degree of block, whether partial or complete, consisted in observing, by means of careful mensuration of tracings, the exact relation of auricular to ventricular beats. We felt that we

Versuchen sehr zu statten. . . . Die ventrikuläre Extraperiode kann kurzer oder länger sein als eine normal Periode, nur ist sie niemals eine compensierende."

[1] "Seine Resulte über die Abklemmung des His'schen Uebergangsbündels beim Hunde stimmen mit meinen Versuchsergebnissen vollständig überein. Es fehlt bei seinen Versuchen nur der Nachwies dass die Kammern automatische schlagen und dass keine Erregungsüberleitung mehr stattfindet."

[2] HERING: Zeitschrift für experimentelle Pathologie und Therapie, 1905, ii, p. 82.

[3] ERLANGER: Zentralblatt für Physiologie, 1905, xix, p. 9; also, Journal of experimental medicine, 1906, xiii, p. 8. ERLANGER and HIRSCHFELDER: Zentralblatt für Physiologie, 1905, xix, p. 270; also, This journal, 1906, xv, p. 153.

were justified in concluding that the block was complete when the auricles and ventricles were beating with perfect regularity, with different rates, and with a relation to one another that was constantly varying; and that the block was partial when the ventricular contractions were less frequent than those of the auricles and when the former bore a constant sequential relation to certain of the latter.

An estimate of the relative values of the two methods for proving the presence or absence of complete heart block from a study of tracings may be formed from the results of the experiments which follow.

Since the term, compensatory pause, has been, and frequently will be, used in this paper it becomes essential to know just what is meant by the term and what is the cause of the phenomenon. By compensatory pause is usually understood the pause which follows an extra systole (usually of the ventricles) induced in the normally beating heart. "The combined duration of the last (*i. e.*, the cycle shortened by the extra systole) spontaneous ventricular cycle and the extra systole following it plus the compensatory pause is (within a small fraction of a second) equal to the duration of two spontaneous ventricular periods."[1] The explanation of the compensatory pause now generally adopted is the following: In the normally beating heart the ventricle is stimulated rhythmically by the impulses which are conducted to it from the auricles. When an extra systole of the ventricle is induced the next of the regularly recurring impulses either falls into the refractory period of the extra contraction or, if it arrives in the ventricle somewhat later, it is subminimal. In either case it fails to elicit a ventricular contraction. Therefore the next auricular impulse causes the ventricle to contract. It has been shown that if the heart rate be reduced by cooling the sinus it may become possible to insert a ventricular extra systole between two natural ventricular contractions. In such a case there is no compensatory pause.

In partial heart block as well as in the heart with cooled sinus, the ventricles beat slowly. But in the former the auricles beat with normal rate, whereas in the latter the rate of the auricles is reduced as well as that of the ventricles. It is therefore impossible to predict from *a priori* reasoning what will occur when a ventricular extra systole is induced in a heart with partial block at the auriculo-ventricular junction. This matter can be decided by experiment only.

[1] HOFMANN: NAGEL'S Handbuch der Physiologie, 1905, i, 241.

Experiments with the Dog's Heart.

General methods. — The various stages of heart block were produced by clamping the auriculo-ventricular bundle of His according to the method described in previous publications. The movements of the heart were recorded by means of tambours. It sufficed to record the movements of the right ventricle alone when the auricles were beating vigorously, for under such circumstances the contractions of the auricles, excepting those occurring during ventricular activity, send distinct waves into the ventricles. When the auricles were not beating with a vigor sufficient to visibly project blood into the ventricles, the movements of the right auricle as well were recorded. As a rule the extra contractions were elicited by means of unipolar induction shocks either of the right ventricle or of the right auricle. In a few experiments the induction shocks were sent through the tissue included in the grasp of the heart clamp. The moment of stimulation was recorded by means of a signal inserted in the primary circuit. The time was recorded in fifths of seconds.

Eight new experiments have been performed. These were all quite protracted so that, as in the earlier experiments, the opportunity was afforded of repeating each observation over and over again in the same and in different experiments.

The reaction of the heart with complete block at the auriculo-ventricular junction to induced extra systoles. a. *The duration of ventricular extra systoles.* — Under this heading it will suffice to state that the results of the writer confirm in every detail those obtained by Hering. Induced extra systoles of the ventricles are never compensatory. They may be a trifle longer or shorter than spontaneous cycles but the differences rarely if ever exceed one-fifth of a second (see Fig. 1).

b. *On the propagation of extra impulses*[1] *through the auriculo-ventricular junction.* — As we have already stated elsewhere, in confirmation of Hering's observations, extra systoles of the auricles or ventricles never elicit corresponding contractions of the unstimulated chambers when the block is complete.

The reaction of the heart with partial block at the auriculo-ventricular junction to induced extra systoles. a. *Ventricular extra systoles.* —

[1] The impulse referred to here and elsewhere in this paper is the one which sweeps through the heart tissue with the contraction wave.

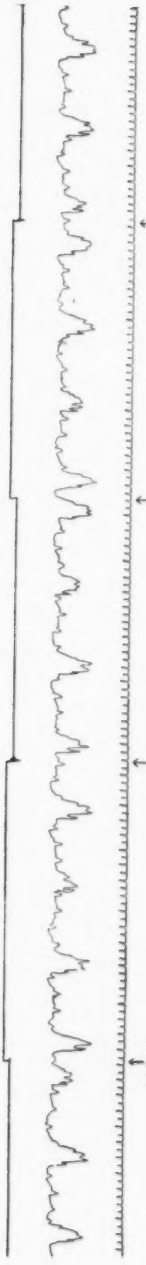

FIGURE 1. — Seven-ninths the original size. Ventricular extra systoles induced during complete heart block (dog). Contractions are marked by down-strokes. The large contractions are ventricular; the smaller contractions, recurring at regular intervals, are auricular. Time in fifths of seconds.

The duration of ventricular extra systoles[1] in partial block, too, as a rule differs but little from that of the ventricular cycles determined by the auricles. The extra period may be a trifle longer or shorter than the uninfluenced ventricular cycles but practically never are they compensatory. This result finds a simple explanation in the obvious tendency for the same number of auricular beats to intervene between the extra contraction and the next uninfluenced ventricular contraction, as intervene between any two uninfluenced ventricular contractions. For example, if in a heart with a partial block with a 3 : 1 or 4 : 1 rhythm (Figs. 2 and 3) a ventricular extra systole is induced in any phase of the cycle, the next following ventricular systole will be caused by that auricular systole which will give to the ventricular extra period a duration which approximates most closely that of three or four auricular beats, respectively. In this there is some variability but the variations are in most instances due to an instability of the block at the time the extra systoles are induced. Thus in a heart that is slowly recovering from a complete block and in which, for example, the rhythm is 3 : 1, extra systoles induced in different stages of the recovery may give the following results: When the extra systole is induced shortly after recovery from the higher stage of block it may rarely result in a temporary return to the preceding stage of block (Fig. 4); but usually the extra period is longer than three auricular beats by such part of an auricular beat as may have been unfinished at the moment the extra systole was induced. But when the extra systole is induced shortly before the rhythm changes to 2 : 1, the extra systole will usually be shorter than three auricular beats by an amount equal to such part of an auricular cycle as may have been completed when the extra

[1] The terms extra systole, extra cycle, and extra period are used interchangeably in this paper.

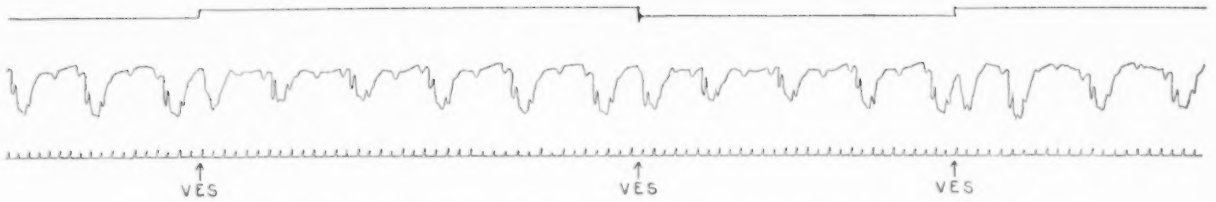

FIGURE 2. — Ventricular extra systoles induced in partial block, rhythm 3 : 1 (dog). Time in fifths of seconds.

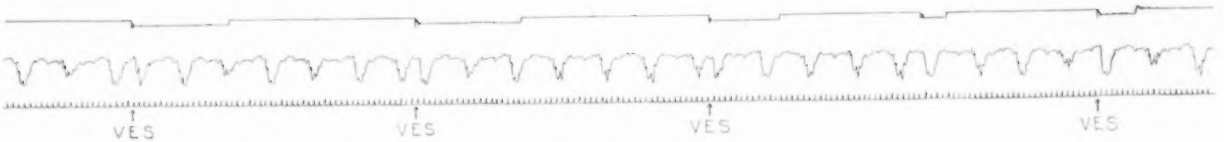

FIGURE 3.— Seven-twelfths the original size. Ventricular extra systoles induced in partial block, rhythm 4 : 1 (dog). Only the break shocks (downstrokes of the signal) are efficient. Time in fifths of seconds.

systole was induced (Fig. 2; also Fig. 5). To any stage between the two instanced the general rule mentioned above applies.

It is therefore obvious that the average duration of ventricular extra systoles induced in any stage of partial block will exactly equal that

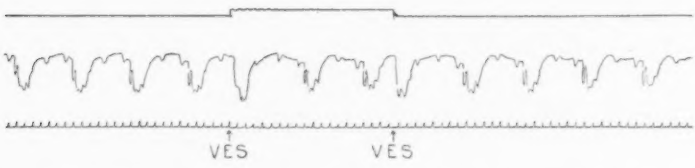

FIGURE 4. — Four-fifths the original size. Ventricular extra systoles induced in partial block shortly after the rhythm has changed from 4 : 1 to 3 : 1 (dog). Time in fifths of seconds.

of the ventricular cycles determined by auricular impulses. When we are dealing with high degrees of partial block (3 : 1 or over) in an animal with a rapid heart rate (in the dog the heart rate often exceeds 120 per minute), the duration of the extra systoles differs so little from that of uninfluenced ventricular cycles that no conclu-

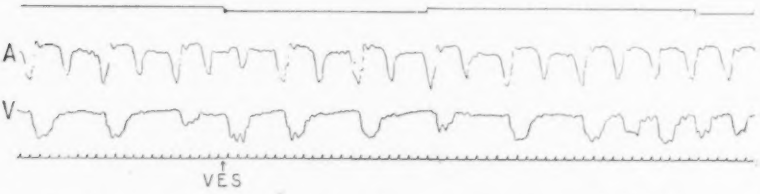

FIGURE 5. — One-half the original size. Ventricular extra systole induced in partial block while the rhythm is 2 : 1 shortly before the rhythm becomes 1 : 1 (dog). A = auricle. V = ventricle. Time in fifths of seconds.

sion can be drawn as to the degree of block, whether partial or complete, from a study of the duration of ventricular extra systoles alone. In such a case the decision can be reached only by means of a study of the relation of ventricular to auricular contractions (see Fig. 6).

b. *The propagation of extra impulses over the auriculo-ventricular junction. — a. Ventricular extra systoles.* — In recent experiments auricular contractions have never been caused by impulses of ventricular extra systoles induced in any stage of partial block. That the impulses of ventricular extra systoles rarely if ever pass the auriculo-ventricular junction in partial block is indicated by the fact that

rhythmic stimulation of the ventricles affects neither the rate nor the rhythm of the auricles.[1]

β. Auricular extra systoles. — Occasionally the impulses of induced auricular extra systoles may elicit ventricular contractions. This occurs, however, only when the induced systole immediately precedes that spontaneous auricular contraction which would have stimulated the ventricles (Fig. 7). This phenomenon is more apt to take place when the heart is about to pass into a lower stage of partial block than when the block is stationary. It is nicely illustrated by those experiments in which, during partial block, the rate of the auricles is increased by artificial means. If the stage of block has just developed from a higher one, the auricular acceleration, if not too great, will be associated with a return to the former stage of block; but if the block is about to pass into a lower stage then, if the degree of block is not changed, the ventricular rate will be increased considerably by the acceleration of the auricles.[2]

c. *The influence of induced auricular extra systoles upon the auriculo-ventricular sequence.* — During recovery from complete block and shortly after the heart has fallen into any stage of partial block, an auricular extra systole may cause a temporary reversion to the next higher degree of partial block (Fig. 8). This effect is most commonly seen where the extra systole just precedes the spontaneous systole that would have elicited a ventricular contraction, and it may last through several ventricular cycles.

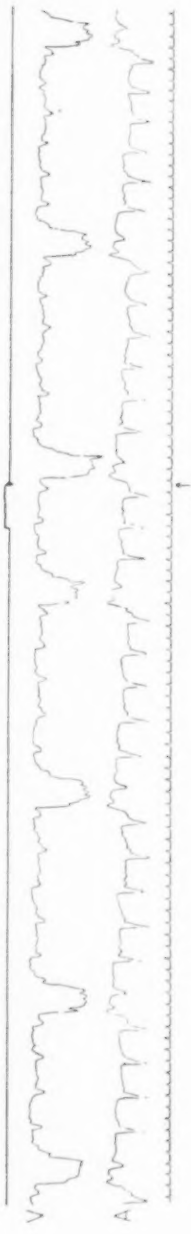

FIGURE 6. — One half the original size. Partial block of an unusually high degree, the rhythm of the successive ventricular cycles being 5 : 1, 6 : 1, 6 : 1, ventricular extra systole of 7 : 1, 7 : 1, (dog). A = auricle. V = ventricle. Time in fifths of seconds.

[1] ERLANGER and HIRSCHFELDER: This journal, 1906, xv, p. 196.

[2] ERLANGER and HIRSCHFELDER: *Loc. cit.*

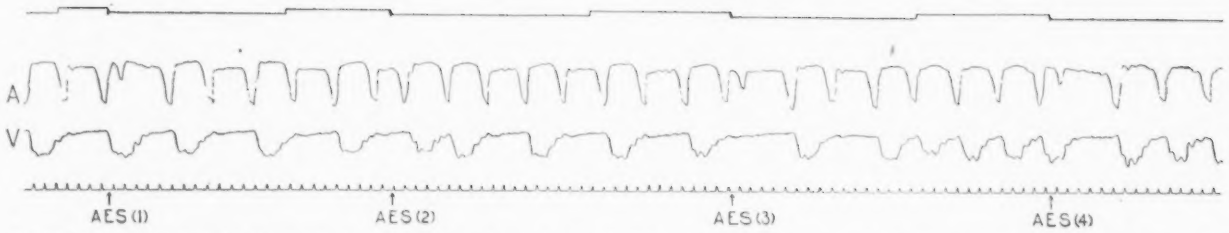

FIGURE 7. — Seven-twelfths the original size. Partial block, rhythm 2 : 1 becoming 1 : 1 at the end of the figure (dog). Showing some of the effects of auricular extra systoles upon the ventricular sequence. AES 1 (auricular extra systole) falls into the ventricles in their refractory phase; it is longer than the normal auricular periods and the next auricular impulse causes the ventricles to contract. AES 2 is induced shortly before the auricular contraction which would have elicited a ventricular contraction; the ventricles respond to it; the extra auricular period is longer than normal and the next auricular impulse elicits a ventricular contraction. AES 3 is induced somewhat earlier than was (2); it fails to stimulate the ventricles. A = auricle. V = ventricle. Time in fifths of seconds.

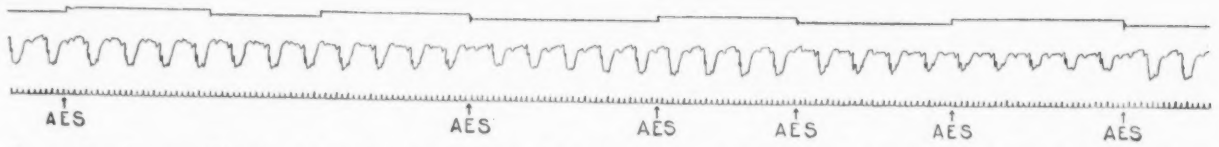

FIGURE 8. — Seven-twelfths the original size. Partial block, rhythm 3 : 1 (dog). Showing one of the results of inducing an auricular extra systole immediately before the auricular contraction which would have elicited a ventricular contraction. Time in fifths of seconds.

On the other hand it not infrequently happens that although the induced extra systole has no immediate influence upon the ventricular rate, it may have a late effect, presumably through the change in the auricular rate which not infrequently follows an auricular extra systole. It was shown by Cushny and Mathews in the dog [1] that an auricular extra period may be considerably longer than the normal auricular periods and that occasionally, after such an extra systole, the normal auricular period may be gradually re-established through several auricular cycles. When this phenomenon is seen during

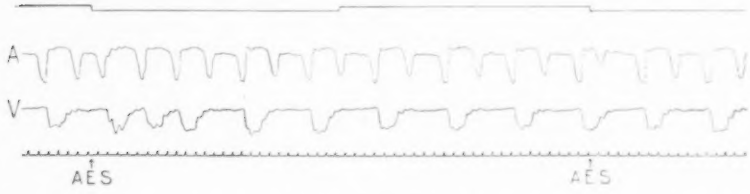

FIGURE 9. — One-half the original size. Partial block, rhythm 2 : 1 (dog). Showing a prolonged auricular extra systole followed by a slightly prolonged spontaneous auricular cycle. During these cycles the rhythm is 1 : 1. A = auricle. V = ventricle. Time in fifths of seconds.

partial heart block a lower degree of heart block may develop as the result of it (for example a 2 : 1 from a 3 : 1 rhythm, or a 1 : 1 from a 2 : 1 rhythm, Fig. 9), and may persist until the auricular rate becomes normal, or, rarely, it may even last some time thereafter. Occasionally, however, an auricular extra systole is followed by a temporary diminution in the degree of block where no after effect upon the auricular rate is detectable.

But whatever may be the reaction of the ventricles to auricular extra systoles induced during partial block, this reaction almost invariably suffices to distinguish the partial block from complete block. For whether or not the auricular extra systoles induce ventricular contractions, they usually alter the regular sequence of auricular beats and therefore of the ventricular beats as well. The variations from the normal of such altered ventricular beats are often not much greater than the variations which result from ventricular extra systoles. But since in complete block auricular extra systoles have no effect whatever upon the duration of ventricular cycles, the small variations which are seen in partial block suffice to distinguish

[1] CUSHNY and MATHEWS: Journal of physiology, 1906, xv, p. 226.

the latter from the former. As a rule the distinction between complete and partial heart block may easily be made by the measurement of tracings. Occasionally, however, the ventricular rate in complete block may be an aliquot part of the auricular rate over long intervals of time, and in such instances the ventricular beats may so follow the auricular beats as to appear to be caused by them. In these rare instances induced auricular extra systoles may serve to unmask the complete block, since they will disturb the relation of the auricular to the constant ventricular beats. But in this connection it should be recalled that under certain conditions, one of which was mentioned above, an auricular extra systole may momentarily increase the existing degree of block and it might, indeed, in the experience of the author, it has, changed a partial block of the 3 : 1 type momentarily into complete block. In such a case the rapid return to the 3 : 1 relation would usually suffice to prove that the block had previously been partial.

The effect on the auriculo-ventricular sequence of varying the auricular rate during partial heart block. — In another place [1] it has been shown that by increasing or diminishing the auricular rate during partial heart block the degree of block may be increased or diminished, respectively. One of the effects of increasing the auricular rate, namely, a change from partial to complete block with stoppage of the ventricles, is shown in Fig. 10. There are two possible explanations of the development of the complete block under these circumstances :

1. It might result from fatigue of the auriculo-ventricular bundle, or

2. It might result from a diminution in the strength of the auricular impulses started in the more rapidly beating auricles.

In regard to the first suggestion, it was assumed in an earlier communication, not without question, however, that fatigue is the cause of the development of complete block under these circumstances. But if the auriculo-ventricular bundle possesses the same properties as other heart muscle (see later), this suggestion cannot stand. Indeed, recent experiments indicate that fatigue is not the cause of the development of complete block under these circumstances. If, as may be seen in Fig. 10, an auricular cycle of approximately normal duration be interposed in a series of accelerated auricular beats, the ventricles, which have been in stoppage, will at

[1] ERLANGER and HIRSCHFELDER : *Loc. cit.*, p. 180.

once respond to it with a contraction. If the stoppage of the ventricles had been the result of fatigue of the auriculo-ventricular bundle it does not seem likely that the bundle would have recovered sufficiently during one normal, or slightly prolonged, auricular cycle to have permitted of such a prompt return to the stage of block which had preceded auricular stimulation. It should however be mentioned that in experiments of this kind it occasionally happens that the stoppage of the ventricles persists for some time after the auricles have been permitted to resume their normal rate. But we have reasons for believing that this result depends, not upon fatigue, but rather upon an accident of the experiment. We believe that the distending ventricle pushes the clamp into a position in which it pulls upon the tissue included in its grasp and thus increases the pressure upon the bundle.

On the irritability of the ventricles in different stages of heart block. — Before a satisfactory explanation can be offered of the cause of partial heart block it will be necessary to know what effect, if any, compression of the auriculo-ventricular bundle may have upon the irritability of the ventricles. The relative irritability of the ventricles in the various stages of partial and in complete block was therefore determined, and by the following method: The lever of the tambour which recorded the movements of the ventricles was arranged in such a way that it opened a primary circuit practically at the moment it returned to its position of rest. The ventricles, which were connected in the secondary circuit, therefore received a break shock practically at the end of

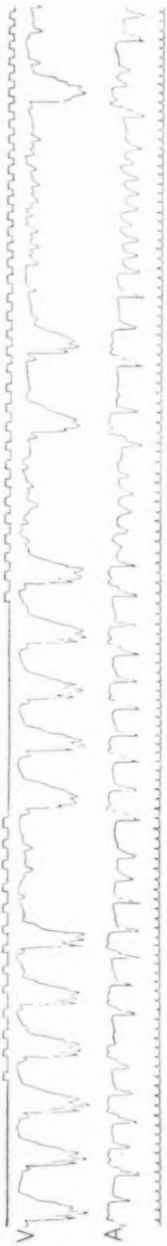

FIGURE 10. — About one-half the original size. Partial block, rhythm 2 : 1 (dog). Showing one effect of increasing the auricular rate. The auricles were stimulated with rhythmic induction shocks. Where the auricle occasionally fails to respond to a shock an auricular period of normal or slightly prolonged duration occurs. The auricular impulse which terminates such periods stimulates the ventricles. A = auricle. V = ventricle. Time in fifths of seconds.

the phase of relaxation. The stimulus was therefore received in the same relative position of the cycle in all stages of block.

Such experiments have shown conclusively that the irritability of the ventricles is not lowered by increasing the degree of block. Indeed, as a rule, the irritability increases slightly but definitely. Thus it may be seen in the following table that, as the degree of block increases, the strength of the minimal stimulus decreases slightly. It is interesting to note that this change in irritability apparently is gradual. Thus the irritability increases slowly when the rhythm changes from 1 : 1 to 2 : 1 and diminishes slowly when the change in rhythm occurs in the other direction.

Does the irritability of the ventricles in heart block increase during each cycle? — It has been conclusively shown in the beating heart of cold-blooded animals that the irritability of the heart muscle increases more or less regularly between contractions. If this occurs in the heart of warm-blooded animals too, we should expect it to be easy of demonstration in the ventricles during complete block since in this condition the ventricular cycles are quite protracted. In order to determine whether or not the irritability does so increase, the following method was first used: Essentially, an attempt was made to find unipolar induction shocks of such a strength that they were subminimal at the beginnings of the cycles but became efficient stimuli as the ends of the cycles were approached. The stimuli were applied to the right ventricle. This method proved to be unsatisfactory. For it was found that within a range of less than 1 mm., with the secondary coil at a distance of 16 cm. from the primary, it was possible to find stimuli which were subminimal or which were effective in almost every part of the cycle. It is obvious that results obtained by such a method could be conclusive neither one way nor the other. As a matter of fact, by this method extra systoles were often obtained early in a cycle when, with the same strength of stimulus, contractions could not be elicited near the close of a cycle. In explanation of such a result we would suggest that an extra systole induced early in a cycle may reduce the irritability of the ventricles for some time thereafter.

In other experiments rhythmic induction shocks at the rate of about 1 in .3 seconds were sent through the tissue included in the grasp of the heart clamp. The position of the secondary coil was varied until an effect upon the rhythm of the ventricles first became perceptible. Analysis of tracings obtained in such experiments made while the

block was complete shows that the usual effect consists in a slight, more or less regular, shortening of the ventricular cycles. The contractions of the ventricles in these places are always apparently caused

TABLE I.

Protocol of an experiment (No. 6, Jan. 19, 1906) to show the variations in the irritability of the ventricles associated with changes in the degree of heart block.

Auriculo-ventricular sequence.	Distance of secondary coil from primary.	Was the stimulus effective.	Auriculo-ventricular sequence.	Distance of secondary coil from primary.	Was the stimulus effective.
	cm.			cm.	
1:1	14	yes	1:1	14	yes
1:1	15	no	1:1	13.5	yes
1:1	15.5	no	2:1	13.5	yes
1:1	15	no	2:1	14	yes
1:1	14.5	yes	2:1	14.5	yes
1:1	14.5	yes	2:1	15	yes
1:1	15	no	2:1	15.5	yes
1:1	15.5	no	2:1	15.5	yes
1:1	16	no	2:1	15.5	yes
2:1	16	no	2:1	15	yes
2:1	15.5	no	2:1	15	yes
2:1	15	yes	1:1	15	yes
2:1	14.5	yes	1:1	15.5	no
2:1	14	yes	1:1	15.5	no
2:1	14	yes	1:1	15.5	no
2:1	14.5	yes	1:1	15	no
2:1	15	yes	1:1	14.5	yes
2:1	15.5	no	2:1	14.5	yes
2:1	15.5	yes	compl. bl.	14.5	yes
1:1	15.5	yes	" "	15	yes
1:1	15.5	no	" "	15.5	yes
1:1	15	no	" "	16	no
1:1	14.5	no			

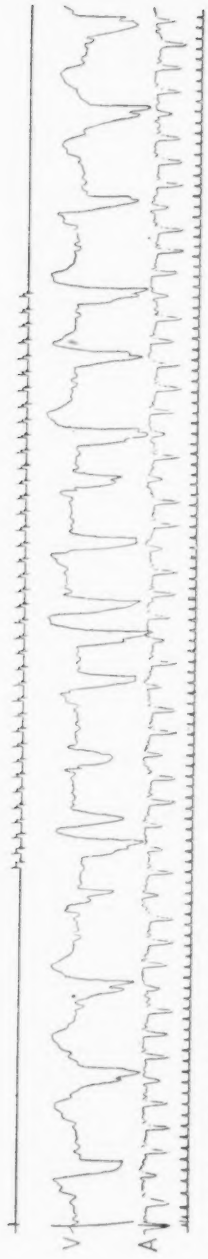

FIGURE 11. — Seven-twelfths the original size. Complete block (dog). Rhythmic stimulation of the tissue in the clamp with almost subminimal induction shocks. A = auricle. V = ventricle. Time in fifths of seconds.

by one of the rhythmically repeated induction shocks (see Fig. 11). It is therefore more than probable that the irritabilty of the ventricles increases during each cycle after the completion of systole.[1] The most conclusive results have been obtained with the heart in complete block, but there is no reason for believing that the same changes in irritability do not occur in partial block as well.[2]

The influence of subminimal stimuli upon the rate of the ventricles in complete block. — Von Basch[3] has shown that subminimal induction shocks increase the irritability of the quiescent cold-blooded heart. For example, this investigator found that a stimulus which was subminimal when repeated at long intervals, excited the heart when it was repeated at shorter intervals. For reasons which will be given later it is important to know if subminimal stimuli will increase the

[1] The author has investigated the changes in the irritability of the ventricles during their long period of rest only. CUSHNY and MATHEWS (*loc. cit.*) have shown that in the normal heart of the dog the irritability of the ventricles increases after the disappearance of the refractory phase. Since these investigators apparently had no especial difficulty in demonstrating this fact, we may be justified in assuming that the increase in irritability is very rapid immediately after the disappearance of the refractory phase, but is slow thereafter.

[2] It is possible that the repeated subminimal induction shocks thrown into the heart in these experiments may have increased the irritability of the ventricles so that they eventually responded to one of the induction shocks which otherwise would have been subminimal. But we think this was not the case because we have not been able to increase the spontaneous rate of the independent ventricles by subjecting them to the action of subminimal stimuli (see below).

[3] VON BASCH: Archiv für Physiologie, 1880, p. 283.

rhythmicity, that is, the rate of the warm-blooded ventricles when they are beating spontaneously. But experiments similar to those described in the preceding subsection have thus far yielded totally negative results.

A Study of Heart Block in Strips of Terrapin's Ventricle.

After it had been shown conclusively, in the dog, that ventricular extra systoles induced in any stage of partial heart block have approximately the same duration as the uninfluenced ventricular cycles, we felt that we might be justified in formulating a theory of heart block if all of the phenomena seen in the warm-blooded heart could be obtained by appropriate treatment of a strip of terrapin's ventricle. It was for this reason that the experiments which follow were performed.

Methods. — A strip of heart muscle, cut from the lower border of the ventricle of the common slider terrapin, was thrown over a Gaskell's clamp in such a way that one half of the strip hung vertically downward over each side of the clamp. Each end of the strip was connected with a lever. Likewise each end of the strip was connected with the terminals of an induction coil. From one of these coils single induction shocks could, when desired, be thrown into the end of the strip with which it was connected. From the other coil rhythmic break shocks, regulated by a Baltzer interrupter, could be thrown into the end of the strip with which it was connected; and in addition isolated shocks, either make or break, could be sent to the same end of the strip by the experimenter as desired. The terminals of both coils passed through a rotating switch by means of which the connections of the coils could be suddenly interchanged from one end of the strip to the other. A piece of rubber dam suspended from the clamp served to insulate the contiguous surfaces of the strip. The heart muscle and clamp were mounted in a moist chamber; but, as an additional precaution, the strip was moistened from time to time with a modified Ringer's mixture containing sodium chloride, 0.7 per cent, and calcium chloride, 0.025 per cent. None of the strips developed a spontaneous rhythm within the time of an experiment.

For the sake of convenience in description, that end of the strip which was being stimulated rhythmically, and which was therefore

contracting rhythmically, will be called the auricular end of the strip, or simply auricle; and the other or following end of the strip will be called the ventricular end, or simply ventricle. The junction of the two ends, of course, lay between the jaws of the heart clamp. By careful regulation of the pressure exerted by the clamp upon the strip any degree of block could readily be obtained.

In this series of experiments the time was marked in seconds. The moments of stimulation of the ventricular end alone were recorded.

The results obtained upon suddenly reversing the direction in which the strip is made to beat. — With a strip prepared in the manner

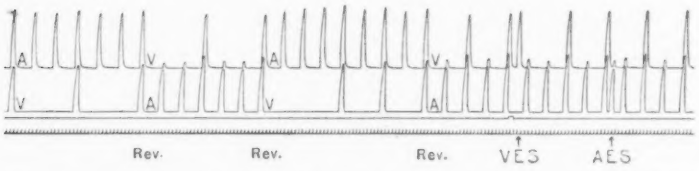

FIGURE 12. — One-third the original size. Showing one of the effects of reversing the direction in which a heart strip in partial block is made to beat. The direction of the beat was changed at the places marked "Rev." Time in seconds. (The small waves on the upper curve were caused by interference of the levers).

described it is possible to parallel practically every result that has been obtained in experiments on heart block in the dog. But before detailing these parallel results it will be necessary to describe the results obtained upon suddenly interchanging the auricular and ventricular ends of a strip. A result which may occasionally be obtained at the beginning of an experiment is illustrated in Fig. 12. In this figure it may be seen that, regardless of the direction in which the strip is made to beat, the degree of block practically remains the same. At the beginning of the figure the rhythm is 3 : 1. Upon reversing the direction of the beat the rhythm remains 3 : 1. At the second reversal there is one cycle of 4 : 1 and then 2 of 2 : 1 whereupon the direction of the beat is again reversed. With this reversal the rhythm remains 2 : 1.

This is the result which we should upon *a priori* grounds expect to obtain in experiments of this kind. But we were surprised to find that in a vast majority of the experiments the degree of block changes with the reversal of the direction in which the strip is made to beat. Thus when the rhythm is 1 : 1 with the strip beating in

one direction, the rhythm may become permanently 2 : 1, 3 : 1, 4 : 1, or the block may become complete when the direction of the beat is reversed (Fig. 13).

No attempt has been made to determine the factors which are responsible for this phenomenon. But it seems that the end of the strip which has been made to contract less is the one which, when stimulated, causes the strip to beat from end to end. This is supported by the observation, illustrated in Fig. 12 (second reversal), that the degree of block is often higher at the moment the reversal

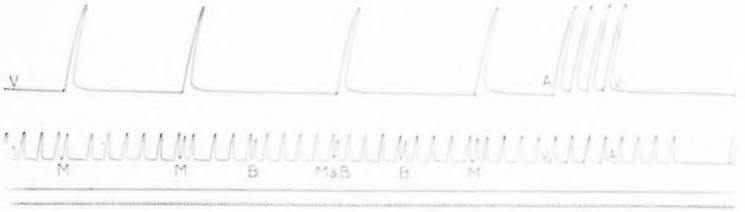

FIGURE 13. — About one-third the original size. Ventricular strip. At the beginning of the tracing the block is complete; none of the rhythmic beats of A stimulate V. Here extra make (M) and extra make plus break (M + B) shocks of A elicit contractions of V, but extra break shocks (B) of A do not affect V. Near the close of the tracing the direction of the beat was reversed: the rhythm thereupon became 1 : 1. Time in seconds.

is made than a short time thereafter. In this case, however, the phenomenon of *treppe* may have played a part.

The results obtained from a study of the effects of extra stimuli. — A study of the transmission of extra stimuli along a strip which reacts in the manner described in the foregoing paragraphs has given the following results:

When the strip is beating in the direction in which the block manifests itself an extra contraction-wave started by mechanical stimulation of the auricle will not, as a rule, pass through the block (see Fig. 14). But under the same circumstances an effective mechanical stimulation of the quiescent or slowly beating ventricle will, as a rule, cause the auricle to contract. When the direction of the beat is reversed and the rhythm becomes 1 : 1, it is still the rule for a wave started by mechanical stimulation of what was formerly the auricle but now is the ventricle, to stop at the block, and for the wave started in what was formerly the ventricle but now is the auricle, to traverse the entire strip. Occasionally, particularly when

electrical stimuli are employed (see below), the contraction wave will traverse the entire strip in both directions. But under such circumstances the delay in the response of the unstimulated half of the strip is always greater when the wave is travelling in the direction in which the block had been complete or partial than when the wave is travelling in the opposite direction. This phenomenon, namely, the transmission of the contraction wave through the block

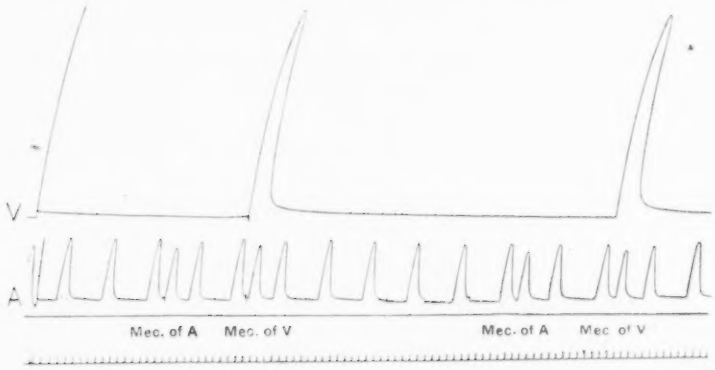

FIGURE 14. — Two-thirds the original size. Ventricular strip. The strip is beating in the direction in which the block is complete (when the direction of the beat was reversed the rhythm became 1 : 1). Mechanical stimulation of A elicits an extra contraction of A but the impulse does not affect V. But effective mechanical stimulations of V elicit contractions of A. Time in seconds.

more rapidly in one direction than in the other, is seen to best advantage when the direction in which the strip is made to beat is reversed almost immediately after the block has disappeared. Thus it may be seen in Fig. 15 that the interval between the contractions of the two halves of the strip is longer when the wave travels in the direction in which the block had previously manifested itself than when the wave travels in the opposite direction.

When electrical extra stimuli are employed, the results obtained may not be exactly the same as those described above. Thus in some experiments in which the rhythm was 2 : 1 or in which the block was complete, an extra make shock or combined make and break shock thrown into the auricle often would cause the ventricle to contract despite the fact that every other, or none, of the rhythmical break shocks which were serving as auricular stimuli resulted in a contraction of the ventricular end of the strip (see

Fig. 13). There can be no doubt but that here the impulse generated by the make was stronger or, at least, more efficient than that of the break. It is interesting to note that in these instances the height of contraction of the end of the strip receiving the extra stimulus was practically the same for both make and break shocks when they fell into the same phase of the auricular cycles. This

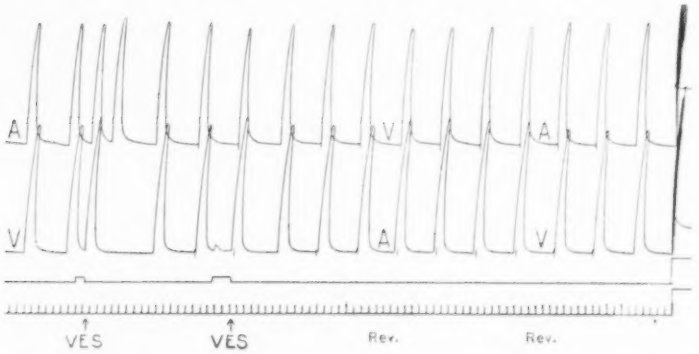

FIGURE 15. — Two-thirds the original size. Ventricular strip showing a difference in the rate of transmission of impulses in either direction through a block which has recovered by enough to allow all rhythmic impulses to pass in both directions. Before this tracing was made the rhythm was 1 : 1 with the strip beating from above downward, *i.e.*, from the end of the strip connected with the upper lever to that connected with the lower; with the strip beating in the opposite direction the block was partial. Time in seconds.

observation indicates that although an efficient stimulus will always elicit a maximal contraction, the impulse which is transmitted through the muscle may vary with the strength of the stimulus.

In this connection it is also interesting to note that when the block is partial or when the partial block has just given way to a 1 : 1 rhythm, a low auricular wave may occasionally fail to elicit a contraction of the entire strip. This is illustrated in Fig. 16.

The facts which have been presented above, permit, in the opinion of the writer, of but one interpretation, namely, that it is the strength of the impulse which determines whether or not it will, after having passed a partial block, serve as an efficient stimulus to the part of the strip beyond the block. It must be admitted that there is no reason for believing that compression of a strip at its middle will give rise to a state which will allow impulses of the same strength to pass more readily in one direction than in the other. But it cannot be denied

that the irritability of the following half of the strip may play a part in this reaction, although there is no reason for believing that in the experiments quoted above irritability is of more than minor significance. This becomes perfectly evident when it is recalled that the rhythmic beats of the auricular end of the strip as well as extra beats of mechanical origin may not cross a block, whereas under the same conditions extra contractions of electrical origin may cross; and that occasional low contractions of the auricular may fail to elicit

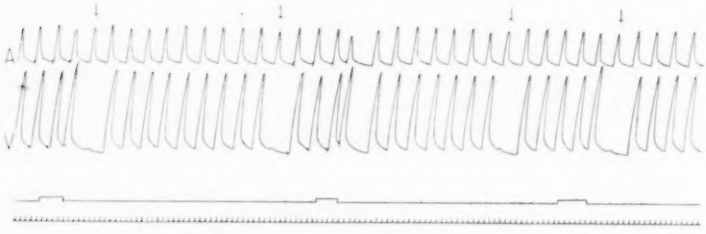

FIGURE 16. — One-half the original size. Ventricular strip. To show that low auricular contractions occasionally fail to elicit ventricular contractions. Time in seconds.

contractions of the ventricular end, whereas the higher waves of contraction as a rule cross the block.

The difference in the interval between the contraction of the two ends of the strip seen where the strip beats from end to end in both directions, but more readily in one than in the other, probably depends upon at least two factors:

a. The weaker impulses may travel more slowly than the stronger, and

b. The latent period of contraction may be longer with feeble than with strong impulses.[1]

The bearing of these observations upon some of the phenomena of the normal heart beat will be considered on a later page. We now shall return to a consideration of the behavior of a strip of ventricular muscle with a partial block toward extra stimuli.

From what has been said before it is evident that whether or not an extra contraction will pass through a block will depend upon the strength of the impulse generated in relation to the degree of block. Of this therefore nothing more need be said than that with proper choice of stimulus and degree of block it is possible to obtain a strip

[1] ENGELMANN: Archiv für die gesammte Physiologie, 1895, lix, p. 318.

which will behave, with regard to the conduction of the wave of contraction, in every way like the heart of the dog in partial block.

Furthermore, extra contractions of the ventricular end of a strip in partial block are, as in the case of the dog's heart, never compensatory. The extra systoles always tend to approximate in duration the uninfluenced ventricular cycles (see Fig. 17). And the variations from this rule are the same in both the heart strip from the terrapin and the heart of the dog.

When, in the case of the strip, the block is complete, the ventricular end does not beat, since ventricular muscle of the terrapin

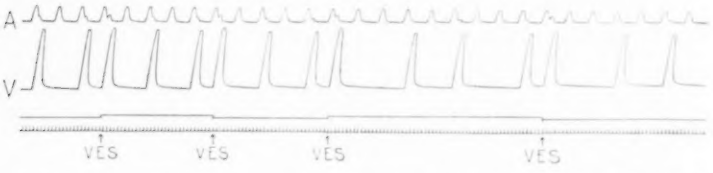

FIGURE 17. — One-third the original size. Ventricular strip. Partial block, rhythm 2 : 1 in the first half and alternating 2 : 1 and 3 : 1 in the second half of the figure. To show the duration of ventricular extra systoles induced during partial block. Time in seconds.

does not possess automatic rhythmicity. But it is well known that when the ventricle of cold-blooded animals is made to beat by treatment with certain saline solutions, extra contractions have approximately the same duration as the spontaneous contractions.

By increasing the rate of contractions of the auricular end of a strip it is possible to increase the existing degree of partial block (Fig. 18). The former sequence, or an improved sequence, is resumed at the moment the original rate of auricular beat is restored.

Finally, it was an easy matter to demonstrate, by a method similar to that which was employed in the case of the dog's heart, that the irritability of the ventricular end of a strip increases between contractions in both partial and complete block. This is merely confirmatory of results obtained by earlier investigators.[1]

THEORETICAL CONSIDERATIONS AND SUMMARY.

All of the observations which have been presented in the foregoing sections of this paper may be satisfactorily explained upon the basis of the theoretical considerations which follow.

[1] The literature is given by HOFMANN: *Loc. cit.*, p. 234.

When the auriculo-ventricular bundle is gradually compressed a stage of partial block is soon reached in which the rhythm is 2 : 1. The irritability of the ventricles is not reduced by compression of the bundle, but it must be assumed that the strength of the auricular impulse which reaches the ventricles is thereby reduced. Presumably the strength of the impulse is reduced by such an amount that the first one which falls into the ventricles after they have contracted is sub-

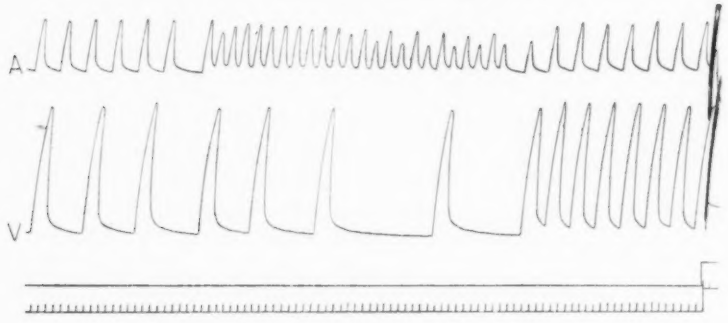

FIGURE 18. — Two-thirds the original size. Ventricular strip. Partial block, rhythm 2 : 1. To show the effect on the degree of block of increasing the auricular rate. When the former auricular rate is restored the rhythm is 1 : 1. Time in seconds.

minimal and the ventricles do not respond to it. Therefore the irritability of the ventricles, which have failed to respond, continues to increase. The next auricular impulse is no stronger than was the preceding one, but it is no longer subminimal for the ventricles in their heightened state of irritability and they therefore respond with a contraction. With this contraction the irritability of the ventricles falls to zero and an interval of time equal to somewhat more than one auricular beat must again elapse before the irritability of the ventricles reaches the level at which the weakened auricular impulse will again become efficient. Higher degrees of partial block may be explained in the same way. In them the impulse which affects the ventricles is still further reduced in strength; and an interval longer than two, or three, or more auricular beats must elapse before the irritability of the ventricles rises to the level at which the enfeebled rhythmic cardiac impulse becomes an efficient stimulus. A glance at the accompanying diagram (Fig. 19) will serve to make this clear. In this diagram, curve I represents the contractions of the auricles (A1, A2, etc.) and ventricles (V1, V2, etc.), and curve II, the assumed

curve of irritability of the ventricles, the horizontal line (O) marking the non-irritable level. In the construction of curve II it has been assumed that the curve of irritability tends to become parallel to the abscissa. The vertical lines extending downward from the beginning of each auricular wave vary in length with the assumed efficiency of the impulse which succeeds in passing through the auriculo-ventricular bundle. Wherever this line intersects the curve of irritability of the ventricles the impulse is effective and the ventricles contract. The influence of the auricular impulse may extend further but its strength is then subminimal. The short horizontal lines at the summits of the waves representing the irritability of the ventricles vary in length with the duration of the intersystolic periods.[1]

Cycles 1 and 2 are drawn to represent the events of the normal heart beat. It may be seen in these cycles that the lines representing the strength of the auricular impulses extend a considerable distance below the curve of irritability of the ventricles. This was so drawn with the intention of indicating that in the normal heart a rather wide variation may occur in the strength of the impulse within the limits of efficiency. After the second cycle, the conductivity of the auriculo-ventricular bundle is gradually diminished. With this the strength of the cardiac impulse which reaches the ventricles diminishes, but the line representing the strength of this impulse still intersects the curve of irritability of the ventricles and elicits a contraction of them; but the im-

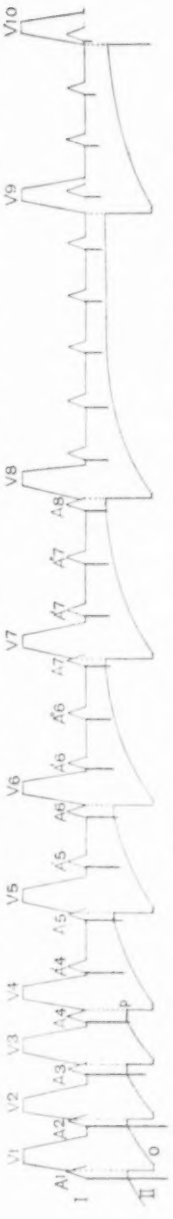

FIGURE 19. — One-half the original size. Diagram to illustrate the genesis of heart block. For description see text.

[1] The intersystolic period is the time which intervenes between the beginning of an auricular contraction and its dependent ventricular contraction. Its variations in heart block in the dog have been described by ERLANGER and HIRSCHFELDER (*loc. cit.*, p. 182). These variations probably depend upon the strength of the auricular impulse which reaches the ventricles in relation to the irritability of the ventricles.

pulse now being weaker than in the earlier cycles, the latent period of the ventricles is longer. The line representing the impulse of A′4 falls short of the curve of ventricular irritability. The ventricles therefore do not contract, and their irritability continues to increase during the rest and reaches a level at which the next auricular impulse (A5), although somewhat feebler than the preceding one, becomes efficient. The intersystolic period may now be shorter than that of the last 1 : 1 cycle, because the increase in the irritability of the ventricles may more than compensate the decrease in the strength of the impulse. This is the condition which obtains in partial block with a 2 : 1 rhythm. In the next ventricular cycle (V5) the conditions (excepting a further reduction in the strength of the auricular impulses) remain practically unchanged. The ventricular cycles marked V6 and V7 are drawn to show the mechanism of the 3 : 1 rhythm. And finally cycles V8, V9, and V10 illustrate what probably happens when the auricular impulses which reach the ventricles are too feeble to act as efficient stimuli. The ventricles then do not beat until their dormant inherent rhythmicity develops sufficiently to cause them to beat independently of the auricles. They will then begin to beat, slowly at first, but with a rate which gradually increases until the comparatively slow rate of the ventricles in complete heart block obtains. This represents the state of affairs in what has been termed relative complete heart block. When the auricular impulses can no longer pass the injured area in the auriculo-ventricular bundle the block becomes absolute.[1]

A review, in the light of this theory, of some of the observations recorded in this paper and in previous papers of the author on heart block, and of some other observations on the phenomenon of conduction across the auriculo-ventricular junction will now be given.

1. It has been shown that a ventricular extra systole induced in any stage of partial block has approximately the same duration as the

[1] It should be noted that it is not necessary, in order to explain the phenomena of heart block caused by a reduction in the conductivity of the auriculo-ventricular bundle, to call upon any other of the properties of heart muscle than those which have been considered above. In most other explanations of heart block the greatest stress has been laid upon a supposed increase in the refractory period at the seat of injury (See HOFMANN: *Loc. cit.*, p. 258). The attempt made by the author to explain his results upon this assumption soon led him into insuperable difficulties. It will not be possible to enter into a consideration of these difficulties here.

uninfluenced ventricular cycles of the same stage of block. This result may be explained by the fact that the extra contraction reduced the irritability of the ventricles to zero, and that therefore the same interval of time must elapse in order that the irritability of the ventricles may again increase to the level at which the auricular impulses become efficient as must elapse after the ventricles have been stimulated by the normal impulse.

2. The development of partial or complete block when the auricular rate is increased may be accounted for in part by the diminution in the strength of the auricular impulses, which presumably is associated with the shortening of the period of rest, and in part by the fact that more of the impulses fall into the ventricles before their irritability reaches the level at which the impulses of the normally beating auricles would have become efficient stimuli.

3. Stimulation of the vagus nerve sometimes causes a block to develop at the auriculo-ventricular junction of the normal heart.[1] But on the other hand, stimulation of this nerve during partial heart block may remove, or diminish the intensity of, the block.[2] There is experimental evidence that stimulation of the vagus nerve neither reduces the irritability of the ventricles[3] nor diminishes the rate of transmission of impulses across the auriculo-ventricular junction.[4] It is, however, certain that vagus stimulation diminishes the rate and strength of auricular beats. In the light of these results a simple explanation of the facts stated above can be made. It is conceivable that under certain circumstances the strength of the auricular impulses in the normal heart might be reduced by such an amount by vagus stimulation that an interval of two or more auricular beats must elapse between each ventricular contraction in order that the irritability of the ventricles may rise to a level at which the weakened auricular

[1] The literature of this subject is given by HUNT and HARRINGTON: Journal of experimental medicine, 1897, ii, p. 723. Here the following significant statement is found: "In a number of cases (of stimulation of the peripheral end of the vagus nerve), however, the ventricle was slowed very decidedly, while the rate of the auricle was but slightly affected."

[2] ERLANGER and HIRSCHFELDER: *Loc. cit.*, p. 182. This has been seen in the cold-blooded heart by GASKELL: Schäfer's Textbook, ii, p. 182.

[3] KRONECKER: Archives internationales de physiologie, 1905, ii, p. 211; here references to the literature will be found.

[4] STASSON: Archives internationales de physiologie, 1905, ii, p. 259. Stasson's experiment is not conclusive because the intervals between the extra contractions of the auricles were longer than the intervals between the normal auricular beats.

impulses can act as efficient stimuli. A partial block would, therefore, result. And it is conceivable that in partial heart block the rate of the auricles may be reduced by vagus stimulation to such an extent that, despite any reduction in the strength of the impulses, a smaller number of auricular beats than normal will elapse in the interval during which the irritability of the ventricles rises to the level at which the auricular impulses become efficient stimuli. The existing degree of block would, therefore, be reduced.

4. It was hoped that this theory would lead to an explanation of the fact that in Stokes-Adams disease an increase in the auricular rate, when the heart block is presumably of the relative form, always precedes, or at least, is synchronous with, the onset of stoppage of the ventricles.[1] In explanation of this association it might be supposed that in the relative form of heart block the part of the auriculo-ventricular bundle which is just below the seat of the injury is the most rhythmical part of the ventricles and therefore determines their inherent rate. And in the relative form of complete heart block it is probable that subminimal auricular impulses constantly arrive in this part of the auriculo-ventricular bundle. The question therefore suggested itself, Might not variations in the strength of such subminimal impulses affect the rate of beat developed by the rhythmical centre of the ventricles? But we have been able positively to influence the rate of the ventricles in complete block by subminimal stimulation of neither the right ventricle nor of the tissue included in the grasp of the clamp. This question, therefore, still remains an open one.

5. We wish also to refer briefly to the light which the experiments on strips of ventricle of the terrapin shed upon the relative strengths of impulses of auricular and ventricular origin. In the case of the dog it has been shown that in partial heart block an auricular extra systole may elicit a contraction of the ventricles if the extra systole occurs shortly before the auricular contraction which would have caused the ventricles to contract. But in recent experiments we have never seen a premature ventricular contraction elicit a contraction of the auricles in partial block. In the opinion of the writer these observations are readily explained upon the assumption that impulses of ventricular origin are weaker than those of auricular origin. Upon this assumption it is also possible to explain the fact that in the normal heart conduction of the retrograde impulse through the auriculo-ventricular junction is less rapid than the conduction of the normal impulse.[2]

[1] ERLANGER: Journal of experimental medicine, 1905, vii, p. 710.

[2] STASSON: *Loc. cit.*

6. In conclusion attention should again be called to the fact that it is possible to simulate by appropriate treatment of a strip of ventricular muscle of the terrapin, all of the phenomena of conduction (normal and abnormal) through the auriculo-ventricular junction of the warm-blooded heart. It is therefore justifiable to conclude that the properties of the auriculo-ventricular bundle of His are, in general, those of ordinary heart muscle.

NOTE ON EQUILIBRIUM IN SOLUTIONS OF PHOSPHATES.

By LAWRENCE J. HENDERSON.

[*From the Laboratory of Biological Chemistry of the Harvard Medical School.*]

I AM greatly indebted to Dr. Otto Folin for the information that Friedenthal's observation upon the reaction of mono-sodium phosphate with methyl orange is incorrect. In his elaborate investigation of the relation between the hydrogen ionization of solutions and their behavior to indicators, Friedenthal found that a decinormal solution of mono-sodium phosphate colors methyl orange red orange.[1]

I have convinced myself in accordance with Folin's statement that both mono-sodium and mono-potassium phosphate are absolutely neutral to methyl orange, and that a fraction of a per cent of free phosphoric acid must be mixed with them in order to obtain an acid reaction with that indicator.[2]

In accordance with this fact it is obvious that the behavior of urine to methyl orange does not prove that disodium phosphate and similar salts must be there present, but only that phosphates in the urine can hardly pass that degree of acidity which is characterized by the presence alone of monobasic salts of phosphoric acid.

I wish to point out that the new facts which I have presented concerning the influence of temperature upon the titratiable acidity of urine and of phosphate solutions do not at all affect comparative results obtained with the aid of Folin's improved method for the determination of urinary acidity,[3] provided that his directions for

[1] FRIEDENTHAL: Zeitschrift für Allgemeine physiologie, 1904, iv. p. 56.

[2] This fact arouses grave doubts of the accuracy of the determination of hydrogen ionization in phosphate solutions reported by Friedenthal. Careful determinations of hydrogen ionization in solutions of $NH_2PO_4 + K_2HPO_4$ are now being conducted in this laboratory.

[3] FOLIN: This journal, 1903, ix, p. 265.

titration immediately after the addition of potassium oxalate are faithfully carried out. Titrations in the presence of potassium oxalate, necessarily carried out at a low temperature, cannot, however, safely be compared with other titrations which have been conducted at the ordinary room temperature.

A STUDY OF THE RELATIONS OF THE INORGANIC SALTS OF THE BLOOD TO THE CONTRACTIONS OF HEART MUSCLE AND SKELETAL MUSCLE.

By E. G. MARTIN.

[*From the Physiological Laboratory of Purdue University, Lafayette, Ind.*]

Introduction.

At the present time practically all physiologists are agreed in assigning to the inorganic salts of the serum, notably those of sodium, calcium, and potassium, a position of prime importance in the proper carrying out of the normal vital functions of the various tissues. This conception has been applied to an especial degree in connection with the rhythmic activity of the heart. Numerous investigations have been made of the influence of salts upon heart activity, from various standpoints, with the result that a large body of facts has been developed illustrating this influence, and demonstrating its existence beyond question. As to its general nature the prevailing view among American physiologists seems to be that enunciated first by Howell in the following words:[1] "I have long been convinced that the inorganic salts of the blood and the liquids of the heart tissue . . . stand in a peculiar and fundamental relation to the initiation of the inner stimulus of the heart contractions."[2] Although the general view that the rôle of the blood salts is to furnish the "inner stimulus" for the heart-beat has found acceptance among these physiologists, the questions as to the particular part played by each constituent, and as to their relative importance, have been the subjects of much discussion, and of pronounced differences of opinion.

[1] Howell: This journal, 1898, ii, p. 40.

[2] After the manuscript of this paper had been submitted for publication the writer was informed by Dr. Howell that in a recent lecture which has not yet appeared in print he developed a view of the functions of the blood salts which does not include among them that of initiating the "inner stimulus" for the heart's contractions.

Thus many observers consider that the cations which are present are the effective agents in arousing the "inner stimulus"; others are inclined to attribute this function rather to the anions. The differences of opinion among those who look upon the cations as of superior importance are confined chiefly to the question of the relative influence of the sodium and calcium ions, it being generally recognized that potassium ions tend to inhibit activity. Loeb[1] and his followers have espoused the cause of sodium, basing their view upon the seeming impossibility of arousing strips of ventricle to spontaneous rhythmic activity unless some sodium salt is brought in contact with the tissue. Howell[1] and his adherents, on the other hand, while not denying the great importance of sodium ions, have been inclined to assign equal if not greater importance to the calcium ions; the chief fact upon which this idea is based is that treatment with precipitants of calcium abolishes all possibility of rhythmic activity, and no procedure restores it except addition of more calcium. Those observers who emphasize the importance of the anions have been led to do so by observation of the very unequal effects produced by different salts of the same metal, as for example the chlorides and bromides of sodium and calcium. Among German physiologists there has been recognition of the essential parts played by the inorganic blood salts in normal heart activity, but the specific assumption of Howell, which assigns to these substances the arousing of the "inner stimulus," has not found favor among them. Langendorff,[2] in a recent paper, reiterates the view proposed earlier by himself and supported by Engelmann, that the true source of the "inner stimulus" is to be found in the products of the heart's own metabolic activity: "Das Lebensprodukt der Zelle ist ihr Erreger." He maintains that Howell's assumption has not been proven to be correct, and considers his own view more in accordance with a just conception of cell physiology. In this same paper Langendorff emphasizes the essential importance of the blood salts, but makes no attempt to assign to them specific functions.

The demonstration of the influence of the inorganic blood salts on the heart-beat suggested at once the probability that the activities of other tissues are affected also by these same substances. Biedermann was the first to call attention to the fact that skeletal muscle tissue ex-

[1] For references see LANGENDORFF: Ergebnisse der Physiologie, 1902, pt. 2, p. 264; also MARTIN: This journal, 1904, xi, p. 103.

[2] LANGENDORFF: Ergebnisse der Physiologie, 1902, pt. 2, p. 322 *et seq.*

hibits spontaneous twitches when immersed in an alkaline solution of a sodium salt. His observations called forth a large number of investigations of the influence of salts on skeletal muscle with the result of developing many interesting and suggestive facts in that connection. The interpretation of these facts has not been a simple task, nor have all investigators been in agreement as to the correctness of the interpretations which have been made. Loeb[1] and Lingle[2] attacked the problem by attempting to homologize skeletal muscle with heart muscle in regard to their reactions to the blood salts. They were in accord with Howell's view that the "inner stimulus" for the heart's activity is aroused by certain of the salts, and looked upon the sodium ions as the effective agents to this end. The twitches given by skeletal muscle in a sodium chloride solution were ascribed by Loeb to a similar action of sodium ions on this tissue; he showed also that the fact that potassium ions tend to inhibit the activities of both tissues is in line with his general view of the similarity of reaction of the two kinds of tissue. When the influence of calcium ions came under consideration difficulty was at once encountered, for Ringer had shown that a small proportion of a calcium salt, such as is distinctly beneficial to heart tissue, inhibits completely the twitching of skeletal muscle in sodium chloride solution. In order to reconcile this difference Loeb and his followers have felt constrained to take the ground that calcium ions, like potassium ions, are really inhibitory to heart tissue as well as to skeletal muscle; the experimental basis for this view rests upon observations of the effect of calcium in concentrated solution, upon which greater stress has been laid than is warranted, in the opinion of many observers, in view of the conspicuously beneficial effect of calcium salts when present in physiological proportions.

Guenther,[3] in a recent paper, has studied the reactions of heart and skeletal muscle to the inorganic blood salts, not with the idea of demonstrating that they are similar, but rather with the purpose of bringing out clearly whatever similarities and dissimilarities exist. He does not make the specific statement that he looks upon the salts as functioning in the heart by arousing the "inner stimulus," although it appears probable from his paper that he inclines toward that view. As the result of his inquiry he concludes that the contractile substance of the heart is not identical with either of the two contractile

[1] Loeb: Festschrift für A Fick, Braunschweig, 1899.

[2] Lingle: This journal, 1900, iv, p. 265.

[3] Guenther: This journal, 1905, xiv, p. 73.

substances which he postulates for skeletal muscle, although in many respects closely similar to that which he designates as the second contractile substance. Guenther's conclusions seem to the author to be the most logical ones which can be drawn from the facts at hand, provided one accepts the view that the salts are the source of the "inner stimulus."

The author was led to undertake this study through his observation of certain phenomena which occurred in connection with another investigation, and which seemed not altogether in accordance with the belief, previously held by him, that Howell's assumption as to the function of the blood salts is the correct one. It was determined, therefore, to attempt the task of bringing together in compact form all the significant data bearing on the problem of the function of the blood salts, and to inquire whether they can be better interpreted according to Howell's assumption or that of Langendorff. Should it appear that Langendorff's position is the stronger it would then be in order to re-examine those having to do with the influence of salts on heart and skeletal muscle in the light of this viewpoint, with the hope that some new suggestion might be forthcoming as to the nature of their influence on these tissues.

The Venous End of the Heart and the "Inner Stimulus."

It has long been established that the normal stimulus for the heart beat arises in the tissues of the venous sinus. The most generally accepted view at the present time, and the one which seems to be strengthened more and more as investigation proceeds, is to the effect that this stimulus in vertebrates arises in certain of the muscular elements of the region. Gaskell[1] has suggested that these particular parts of the tissue remain throughout life in what is practically their embryonic condition, preserving as one of their inherent qualities the essentially embryonic trait of rhythmicity. Inasmuch as the normal stimulus for the heart's activity arises in the tissues of the sinus, it is evident that if the salts are the effective agents in arousing this stimulus their influence must be exerted at that point, so that direct evidence as to whether or not the salts do so function must be sought through studies of the sinus itself. Investigations into the reactions of other heart tissues with salts, ventricular strips for example, are to be looked upon as furnishing only indirect evidence in regard to this

[1] Gaskell: Schäfer's Text-book of Physiology, 1900, ii, p. 180.

question, by means of the light they may shed on the general relations of salts to rhythmic tissues. Only one investigation undertaken with the avowed purpose of studying the influence of salts on the rhythmic activity of sinus tissue is known to the author; that is the one published by Howell[1] in 1898. In that paper Howell emphasized the recently discovered facts of the remarkable power of solutions of the inorganic salts of the blood to sustain the beat of the heart; and interpreted them as indicating that such solutions are thus efficient because among their constituents is to be found the normal source of the "inner stimulus" for the heart's rhythmic activity. Howell himself, on the basis of all the evidence at his command, assigned that function to the calcium ions. Among other observations the fact was brought out that strips from the vena cava of the turtle may continue active for as much as fifty hours in baths containing only sodium, calcium, and potassium salts in solution. A point in that connection, and to the author's mind of great significance, is that in none of Howell's experiments did the strips continue vigorously active for more than five hours in solutions in which the proportions of the salts present were the same as in normal serum, and in order to secure his long-continued series he was obliged to increase the concentration of the calcium salt in the solution from time to time. Now every one who has worked much with turtles' hearts must have observed, as the author has repeatedly, that in hearts which have been cut completely from the body and placed in moist air the venous portion frequently continues to beat for twenty-four to thirty hours after the isolation of the organ; that is to say, the presence about the heart of a pure solution of the blood salts in their proportion as found in serum is the reverse of beneficial to the activity of its venous tissues. Howell looked upon the increased activity which followed increases in the calcium concentration in his experiments as indicating a direct stimulating function on the part of the calcium; an observation of the author's, however, seems to make room for a different interpretation of this effect. A study was being made of the effect upon rhythmic tissues of keeping the salt solutions in which they were immersed saturated with air. The turtles at the author's disposal were not large enough to enable him to obtain records from strips of the vena cava such as Howell used, so the method was adopted of isolating the auricle with an adhering portion of the vein, and obtaining records of the auricular contractions. Although it was recognized

[1] HOWELL: *Loc. cit.*, p. 40.

that this procedure would not give entirely conclusive data as to the behavior of sinus tissue under the conditions studied, it was felt, nevertheless, that inasmuch as the auricle is closely related physiologically to the sinus, observations of the combined behavior of sinus and auricle under any given condition could be looked upon as indicating the probable behavior of the isolated sinus under corresponding conditions. It is, however, desirable to check the results obtained by this method with observations upon strips of vena cava such as were studied by Howell. Two strips of tissue from the same heart were used; both were immersed in normal Ringer's solution (sodium chloride 0.7 per cent, calcium chloride 0.026 per cent, potassium chloride 0.04 per cent); through one of the baths a constant stream of air was kept bubbling. The results of a particularly instructive experiment were as follows: the sinus auricle strip immersed in Ringer's solution, through which no air was bubbling, continued active for a total of eight hours, although during the latter half of this time the beats were very feeble; the companion strip in Ringer's solution, through which air was bubbling, gave the following history: during approximately seventeen hours vigorous, although gradually diminishing contractions were given; at the end of that time the rubber outlet tube leading from the vessel in which the strip was hanging sprung a leak and the Ringer's solution drained off; this happened during the night and was not discovered for five hours. No evil results followed the withdrawal of the bath, however, for when first observed the strip was beating better than it had been when the liquid drained away. The vessel was at once refilled with Ringer's solution through which air was passed as before. The strip continued to beat, again with gradually diminishing vigor, for twelve hours, when the air supply was cut off; in five minutes the height of contraction as registered on the kymograph fell from two millimetres to less than one-half millimetre. During the next thirty-six hours the strip remained alive, executing feeble beats which barely show on the record; the Ringer's solution was then drawn off, and within fifteen minutes after the exposure to the air the strip had resumed beating with sufficient vigor to give a height of contraction on the record of four millimetres. A fresh Ringer's solution was at once brought around the strip and air passed through it in the usual way; the gradually diminishing series continued this time for ten hours, after which the strip gave no more visible beats. Of the eighty hours during which the strip remained alive after isolation thirty-nine were in Ringer's solution through

which air was bubbling, thirty-six in Ringer's solution through which no air was bubbling, and five in air. This strip gave contractions vigorous enough to move the recording lever during forty-four hours in air and air-saturated Ringer's mixture, as against four hours for the companion strip in ordinary Ringer's mixture. This observation seems to show beyond question that in sinus-auricle tissue a sufficient oxygen supply is indispensable to long-continued activity, and that the rapid exhaustion in ordinary Ringer's solution is to be explained as due to insufficient oxygen rather than to a decrease in the effectiveness of the "inner stimulus." Howell's observation of the beneficial effect of increased doses of a calcium salt can be satisfactorily explained, it seems to the author, in the light of this pronounced influence of oxygen, upon the assumption recently advanced by him,[1] which assigns to calcium ions the function of improving the power of the tissue to make use of oxygen, especially when that substance is present in meagre amount. Whether the author's suggestion as to the function of calcium be accepted or not, he is nevertheless convinced that his results, as reported above, tend to impair the force of Howell's argument in favor of the directly stimulating function of calcium. Howell, holding the accepted view that in experiments on cold-blooded hearts no special care need be exercised with regard to the oxygen supply, very naturally concluded that symptoms of exhaustion on the part of a strip of venous tissue in a Ringer's solution were indications of the failure of the "inner stimulus." When the beneficial effect of an increased calcium concentration was observed the equally natural conclusion was drawn that the calcium supplies this stimulus. Now that the author has shown that exhaustion in ordinary Ringer's solution is due to lack of oxygen, and not at all to failure of the "inner stimulus" which may continue effective for as much as eighty hours in a solution of uniform calcium content, this one line of positive evidence in favor of the directly stimulating function of calcium seems to have lost its force. With regard to such other evidence as has been advanced in support of Howell's assumption, that author himself states explicitly[2] that none of it is claimed to give indisputable proof of his position, but rather that his view affords the most satisfactory explanation yet offered for the proven fact of the vital importance of the inorganic blood salts.

The following seems to the author to be a fair statement of the re-

[1] MARTIN: This journal, 1906, xv, p. 316.

[2] HOWELL: *Loc. cit.*, p. 52.

lations of venous tissue to the blood salts so far as they are at present known. In the first place, inasmuch as such tissue continues to beat for a considerable time, twenty-four to thirty hours, when isolated from the body and left entirely without treatment, it is evident that it contains within itself all the conditions necessary for rhythmic activity, including the source of the "inner stimulus." If, however, venous tissue is to be immersed in a liquid, and rhythmic activity maintained, it is necessary that the bath contain sodium and calcium salts in about their proportion as found in serum, and desirable that a small proportion of a potassium salt be also present; it is also essential, if vigorous contractions are to continue for any length of time in such a solution, that sufficient oxygen for the needs of the tissue be supplied. A deficiency of oxygen may, however, be largely compensated by increasing the concentration of the calcium salt in the bath. It appears to be true that venous heart tissue will remain irritable after isolation several times longer in a suitable inorganic solution than it will in moist air — in the author's experiments eighty hours in the one case, and thirty in the other — but it seems to be equally true that this tissue is not in its best condition for activity in the most favorable inorganic solutions, inasmuch as transfer from such a bath to moist air is nearly always followed by improvement in beat. It should be borne in mind that an environment of air is much more foreign to the tissue than is a Ringer's solution, so that the earlier cessation of irritability in the former case is not necessarily proof that the salts of the solution are positive agents in preserving irritability. Finally, the presence within the tissue of soluble calcium salts has been shown to be essential to the existence of rhythmicity. While these facts are undoubtedly in harmony with Howell's assumption, they are equally so with that of Langendorff, inasmuch as they prove nothing more than he freely admits, namely, that certain of the blood salts are in some way necessary to activity. If, as Langendorff claims, his position is logically the stronger, and the burden of proof rests with the believers in Howell's theory, it must probably be granted that so far as the venous part of the heart is concerned the evidence thus far advanced is inadequate for the establishment of the latter.

The Ventricle and the "Inner Stimulus."

When the venous part of the heart was under consideration a tissue was being dealt with which normally exhibits spontaneous rhythmicity

when immersed in the blood itself, or in a solution of its inorganic constituents, or when removed entirely from solution and placed in moist air. When we turn to the ventricle, however, we encounter one which is spontaneously rhythmical under none of these precise conditions, yet which under certain other definite conditions readily becomes so. It is just on this account that studies of the ventricle are so promising; observations of the methods by which a tissue which is normally non-rhythmic is made highly rhythmical can hardly fail to be very suggestive of the conditions upon which automatic rhythmicity depends.

A good method of attacking the problem of the source of activity in the ventricle is by inquiring why it is not spontaneously rhythmical; is it because of the presence of some inhibitory agent which interferes with the development of contractions, or is it because the fundamental conditions of rhythmicity are not fulfilled? Howell,[1] in his well-known analysis of the influence of the blood salts on the heart, suggested that the initial standstill of the ventricle is due to the inhibitory actions of the potassium ions in the tissue; according to this idea the absence of spontaneous rhythmic beats in a freshly isolated ventricle is an inhibition effect displayed in a tissue in which are present all the conditions for automatic activity, requiring only release from the inhibitory influence to become at once effective. The author, in a previous paper,[2] described phenomena which seemed to him to indicate that the standstill of the isolated ventricle is something deeper than a pure inhibition — that it is also due to a lack of one or more of the factors upon which the development of spontaneous activity depends. At that time he held the view that diffusible calcium ions are the factors lacking, and advanced some evidence in support of the idea that the development of spontaneous contractions in a ventricular strip goes hand in hand with the accumulation of diffusible calcium ions within it. A number of the author's recent observations have seemed to indicate that the diffusible calcium ions, instead of being the direct agents in arousing the tissue to activity, function indirectly to that end. One of the observations upon which the writer based his former conclusion was that a preliminary bath of a calcium-containing liquid shortens very markedly the latent period of a freshly isolated ventricular strip in 0.7 per cent sodium chloride solution. He was led to his present conclusion, that the calcium

[1] Howell: This journal, 1901, vi, p. 206.

[2] Martin: *Ibid.*, 1904, xi, p. 103.

function is not a direct stimulation, by the observation that a freshly isolated strip of ventricle immersed in $\frac{n}{8}$ sodium chloride saturated with carbon dioxide begins to beat automatically without any latent period whatever, other than the brief one which is consumed in preparing the strip.[1] The contractions which are given in a carbon dioxide-saturated saline are feeble, not more than one-third the normal height, and exhaustion comes on rapidly,[2] but if after the first half-dozen beats a stream of air be passed through the solution the extent of contraction will shortly increase and a typical series can be gotten under way within three or four minutes after the first immersion. The author is unable to suggest for this phenomenon any better explanation than the assumption that the solution saturated with carbon dioxide exerts a directly stimulating influence upon the tissue, such as ordinary saline solution does not exert. This assumption is supported by the additional observation that passing a stream of carbon dioxide into a sodium chloride solution, in which is a strip in its latent period, is followed promptly by rhythmical contractions, no matter at what stage of the latent period the gas is introduced. This prompt response to a carbon dioxide-saturated saline resembles a direct stimulation effect much more markedly than does the response to treatment with calcium, where even under the best conditions there is a latent period of several minutes. The author has always found difficulty in reconciling the occurrence of this latent period with a conception which makes calcium a source of direct stimulation; to a still greater degree has this difficulty presented itself in connection with arguments in favor of the directly stimulating influence of sodium ions. In order to explain the delayed response it has been necessary to assume, as Howell did, an inhibitory influence of some sort, which must be overcome before the stimulating agents can produce response. Inasmuch as treatment with carbon dioxide-saturated saline is followed by immediate activity, whatever inhibitory agents may be present in the tissue are evidently not in such condition as to interfere with the effectiveness of this stimulant, and it is therefore fully as reasonable to suppose that the slow response to calcium and sodium is due to the fact that they are not direct stimulants, as that their stimulating action is held in abeyance by an inhibitory agent.

[1] NEWMAN: This journal, 1906, xv, p. 379, has recently called attention to a similar "primary stimulation" seen in the heart of Limulus when its ganglion is brought under the influence of carbon dioxide.

[2] See DIVINE: Zeitschrift für Biologie, 1905, xlvii, p. 335.

The fact that only feeble beats are given by strips in the carbon dioxide-saturated medium is no more than is to be expected when one considers the necessarily suffocating effect of saturation with carbon dioxide. If it were possible to apply this stimulus experimentally in the same intra-molecular fashion as Langendorff assumes the normal one to be applied, there would then be no hindrance to that free access of oxygen upon which, as the writer recently showed,[1] depends the execution of vigorous contractions; so the fact that the beats which follow this treatment are feeble is to be attributed rather to the limitations of the method than to the ineffectiveness of the stimulus.

In this treatment with carbon dioxide-saturated saline the actual experimental application of Langendorff's assumed "inner stimulus," namely, the products of the tissue's own activity, is more nearly approached than by any other procedure known to the author, and the conspicuous effectiveness of this very crude partial imitation of that sort of stimulus seems to afford a rather strong indication of the correctness of Langendorff's view; so that to the mind of the writer the evidence now at hand as to the source of the "inner stimulus" for the ventricle is in favor of the conception that it is furnished by certain products of the tissue's own metabolic activity.

At the close of the discussion upon the source of the stimulus for the activity of the venous end of the heart, the opinion was expressed that, inasmuch as convincing evidence to the contrary is wanting, it must be conceded that the weight of probability is upon the side of Langendorff's assumption. The effort to throw additional light upon the question by consideration of the conditions of rhythmicity in the ventricle has seemed to strengthen that position by bringing out experimental data which may be interpreted as affording direct evidence in support of it. It seems proper to the author, then, to adopt this conclusion provisionally, and to proceed, as was suggested at the outset, to a re-examination of the relations of the inorganic blood salts to the activities of heart and skeletal muscle in the light of it, with the purpose of assigning to them, if possible, rôles which shall be in harmony with it, and at the same time in accordance with all facts now known which have bearing. The order of consideration will be: 1, the isolated ventricle; 2, the entire heart; 3, skeletal muscle.

[1] MARTIN: This journal, 1906, xv, p. 303.

THE INFLUENCE OF SALTS ON THE VENTRICLE.

The first questions which come up for discussion under this head are as to the nature of the "initial standstill" and the part played by calcium and sodium ions in arousing the tissue to activity. The following hypothesis seems to the author to explain these points satisfactorily, while at the same time it agrees with all the observations which have been thus far made in this connection. It is assumed that spontaneous rhythmicity in ventricular tissue depends upon two factors: 1, the presence within it of certain definite products of its own metabolic activity, one of which may be carbon dioxide, these constituting the "inner stimulus" of Langendorff; 2, the presence of diffusible calcium ions whose chief function is to promote those reactions between the tissue and oxygen which are essential to its activity. The normal non-automaticity of the ventricle is explained in part by the author's former assumption [1] that its calcium content is in such a combination with the tissue substance that it is not diffusible, and so is not active except when, as the result of the application of an external stimulus, enough of it is changed from the inactive to a diffusible active combination for the requirements of that particular response; and in part also by the supposition that the metabolic activity of the tissue, which depends upon the presence of diffusible calcium ions, is normally too restricted to produce substances which will suffice as stimuli to automatic contractions. The initial standstill of the isolated ventricle being thus accounted for, it is in order to attempt to apply these same assumptions to the explanation of the different experimental methods of arousing ventricle strips to rhythmic activity. Let us begin with a consideration of the method of a preliminary calcium-containing bath followed by $\frac{n}{8}$ sodium chloride. This method, as was shown by the writer,[2] arouses a strip to activity after a short but distinct latent period. According to the hypothesis here being developed the course of events in a strip under these conditions would be about as follows: the calcium-containing bath would yield an abundant supply of diffusible calcium ions; under their influence the tissue would react freely with oxygen, entering upon metabolic changes which, while not taking the form of contractions, might be supposed to yield meta-

[1] MARTIN: This journal, 1904, xi, p. 133.
[2] *Ibid.*, p. 107.

bolic products of suitable form, and in due time in sufficient quantity, to serve as an efficient stimulus to automatic contractions; when these substances have accumulated thus to the requisite degree the conditions of rhythmicity are fulfilled and activity commences. Fresh strips immersed directly in $\frac{n}{8}$ sodium chloride show a longer latent period than in the case just discussed. In this medium no diffusible ions are supplied directly, and to harmonize this method of inducing beats with his view that such ions are essential to activity the author made the assumption [1] that there is a gradual conversion of the calcium-containing compounds of the tissue from their normal form, in which the calcium is indiffusible, to one in which it is diffusible. The tentative suggestion was advanced that this conversion might occur as the result of the action of sodium salts upon the tissue.[2] Continued study along this line has strengthened the author's belief in the possible correctness of this idea to such a degree that he feels justified in including as a definite part of his hypothesis, the assumption that the conversion of calcium to its diffusible form takes place as the result of the mass action [3] of sodium ions. During the latent period of a strip in $\frac{n}{8}$ sodium chloride there would occur then: first the reaction between sodium ions and the calcium tissue compounds, by which the calcium passes over into a combination in which it is diffusible; then the reaction between the tissue and oxygen under the influence of the diffusible calcium ions, with the resulting accumulation of stimulating metabolic products; and finally the onset of rhythmic contractions under the stimulus of these products. A longer latent period would be expected to occur under these conditions than under those considered just previously, because here more steps are involved in the production of the essential conditions of rhythmicity. When fresh strips are immersed in $\frac{n}{8}$ sodium chloride saturated with carbon dioxide they are brought at once into contact with a substance, which, according to the writer's hypothesis, is nearly enough like the normal "inner stimulus" to serve in place of it. Since the strip is thus provided at once with an efficient stimulus, and does not have to wait for the development of one according

[1] MARTIN: *Loc. cit.*, p. 133.

[2] *Ibid.*, p. 133.

[3] The term "mass action," as used here and subsequently in this paper is not intended to be understood strictly in its technical chemical sense, but merely as indicating an action which requires the presence of large numbers of ions for its performance.

to the roundabout method outlined above, it is to be expected that it will begin to show contractions very soon after immersion in the saline bath. The contractions which are given under this stimulation are feeble, representing, therefore, a meagre oxidation which, in turn, requires only a small supply of diffusible calcium ions such as would be available immediately after the immersion in the sodium chloride solution.

It will be observed that, according to the terms of the hypothesis under consideration, which assigns to sodium ions only the function of inducing, by their mass action, the conversion of calcium tissue compounds into forms in which the calcium ions are diffusible, it should be possible to dispense with the presence of sodium ions altogether, provided diffusible calcium ions be directly furnished. What affords to the author's mind one of the strongest arguments in favor of his hypothesis is his observation that by proper procedure along the lines it suggests a fresh ventricular strip can be made to give long series of spontaneous contractions without coming into contact with any sodium salt whatever, at any stage of the experiment. Before the method of obtaining this result is described it is desired to call attention to some of the difficulties attending the successful application of diffusible calcium ions; difficulties to which are to be attributed, in all probability, the widely held view that rhythmic activity cannot be induced in ventricular tissue unless treatment with some sodium salt is included in the procedure. If the strip be immersed in a calcium chloride solution isotonic with serum, the evil effects of an overdose of calcium are bound to appear immediately, and make the manifestation of a normal calcium effect impossible. If, on the other hand, the strip be placed in a calcium chloride solution of physiological concentration (0.03 per cent) conditions favoring extensive osmotic disturbances are presented, in the face of which normal responses are not to be expected. There has been full recognition of these difficulties, and attempts have been made to avoid both of them at once by using as bathing media solutions containing a calcium salt in physiological concentration, and made isotonic with serum by the addition of some "indifferent" substance. This method is open to the very serious objection that it involves the assumption of physiological indifference for whatever substance is used in making the solution isotonic. The substances which have thus far been suggested from *a priori* reasons as likely to be indifferent — sugar, urea, lithium chloride — have without exception, when

subjected to the test of experiment, given abundant indication of positive influence upon the tissue of one sort or another, so that conclusions drawn from experiments made in this manner cannot be wholly convincing. With all these points in mind it seemed to the author that his best chance of success lay in the use of pure aqueous solutions of calcium chloride of approximately the concentration of the calcium salts in serum. By the employment of such solutions the well-known injurious effects of concentrated solutions of calcium salts are avoided, and at the same time there is not that introduction of unknown influences which inevitably accompanies the use of so-called indifferent substances to make the solution isotonic. There is brought in, to be sure, an extraneous factor to be reckoned with in the hypotonicity of the solution; but the author's experience has been that the abnormal effects resulting from the use of hypotonic solutions are minimized if instead of immersing the strips in them they be simply moistened with a few drops thereof. In order, however, to be sure that the phenomena which follow the use of dilute calcium chloride solutions are due to the calcium ions and not to the influence of hypotonicity, it is essential that the effects of hypotonicity be known. Carlson[1] has recently studied the responses of Limulus hearts and tortoise auricular tissues to such solutions, and has shown that in them the influence of these solutions is uniformly augmentor. The author has observed the same thing in strips of turtle ventricle; but the stimulating influence of hypotonicity, pure and simple, as exhibited in strips treated according to the author's method is wholly transient in character, and there is no indication of a tendency to induce long series of contractions such as appears when calcium ions are present. An experiment illustrating this difference will be quoted presently; it is desired first to give a detailed description of the experiments in which automatic activity was induced in ventricle strips without the application of sodium ions.

A strip of turtle's ventricle, cut in the usual way, was placed directly, without rinsing or other treatment, in a moist chamber and attached to a recording lever. An abundant stream of carbon dioxide was then passed through the moist chamber, completely replacing the usual atmospheric environment; the idea back of this procedure was that, by thus introducing a stimulating medium directly, one of the two essential conditions of rhythmicity postulated by the author

[1] CARLSON: This journal, 1906, xv, p. 357.

would be fulfilled immediately, so that the only additional factor to be supplied would be the diffusible calcium ions. After the strip had been in the carbon dioxide atmosphere for four or five minutes, and before any application of the calcium chloride solution to it, a stream of air was passed into the moist chamber to sweep out the carbon dioxide; this was done because previous experience had shown that no vigorous contractions would be given in a carbon dioxide atmosphere. As soon as the carbon dioxide had been replaced by air the strip was moistened with four to ten drops of 0.1 per cent calcium chloride solution, applied by dropping it upon the strip from above. Usually the application of the calcium-containing solution was followed promptly by a few vigorous contractions, but in order to establish a long series the writer found it necessary to repeat the treatment — carbon dioxide, air, calcium chloride solution — three or four times in succession. The series being once established would continue for a considerable time — from two and a half to nine hours — without further attention. Some of the details of these series will be discussed presently; at this point the author wishes to emphasize the fact that we have here true spontaneous rhythmicity induced in ventricular tissue without any application of sodium ions whatever. In the face of this observation it is difficult to see how theories based upon the absolute necessity of the external application of sodium salts can be maintained. Occasional instances are on record of strips of ventricle which exhibited spontaneous rhythmic contractions without the application of salt solutions of any sort to them, but all observers are agreed that these are exceptional, and that they represent some abnormal condition of the tissue. Leaving all such out of account, the numerous attempts which have been made to demonstrate that the external application of sodium ions is unnecessary to the development of automatic rhythmicity have hitherto given only negative results, similar negative results have followed all procedures employed by the writer except such as correspond in principle with the successful experiments described above. For that reason he feels that the fact that the one method outlined was successful is strong indication of the correctness of the principle upon which it is based.

In the hypothesis proposed by the author to explain the development of rhythmicity in strips immersed in salt solutions, the position was taken that if diffusible calcium ions be supplied the "inner stimulus" will be developed indirectly through the vigorous oxidative

metabolisms thereby promoted; now it may be asked why, in the experiment just described, in which, likewise, diffusible calcium ions are furnished, the treatment with carbon dioxide might not be omitted, with the expectation that there would occur here a similar indirect development of an effective "inner stimulus." The author would reply by stating that, according to his hypothesis, such an expectation would be theoretically sound, provided no extraneous factors are introduced by the difference between an environment of salt solution on one hand and of air on the other. As a matter of fact there is, in a strip suspended in air and treated with a calcium-containing solution, a strong tendency to go into that condition of extreme tone known as calcium rigor, which, according to a recent suggestion of the author's,[1] is due to over-oxidation. It is on account of this tendency that the use of carbon dioxide is necessary; the theoretical justification for its employment is on two grounds; in the first place it supplies a stimulus directly, thus avoiding the necessity of applying calcium ions for the purpose of developing one indirectly, and so enables the use of the calcium-containing solution to be kept at lowest terms; in the second place, since the tissue is thoroughly saturated with carbon dioxide, and the air admitted to it only just at the moment of the application of the calcium ions, the consumption of oxygen is to a certain extent interfered with, and there is not so great tendency for it to become excessive. Comparison of experiments in which carbon dioxide was and was not used brings out clearly its restraining influence on the tendency of the strip to go into "calcium rigor."

In a single case the writer obtained a result which is interesting as tending to confirm his idea that carbon dioxide simulates the normal "inner stimulus." A strip of ventricle was suspended in a moist chamber and stimulated by induction shocks at intervals of four seconds for an hour and twenty minutes. It was then moistened with a dilute calcium chloride solution and after a time began to contract rhythmically. The length of the rhythmic series did not exceed one hour and the number of contractions was only about one hundred seventy-five, but it was, nevertheless, an undoubted instance of the development of rhythmicity without the application of sodium ions. The point of interest involved is that in this experiment no carbon dioxide was applied, but in its stead there were present, presumably, products of the tissue's metabolism in considerable

[1] MARTIN: This journal, 1906, xv, p. 317.

quantity, since there were present no means by which such products could be swept out. Inasmuch as this strip gave a response similar to that of others after treatment with carbon dioxide, it is at least possible to suppose that the similarity of response indicates a similarity of influence between carbon dioxide on the one hand and the tissue's metabolic products on the other.

Reference was made in a preceding paragraph to experiments which demonstrate that the peculiar stimulating influence of a hypotonic calcium chloride solution is due to the calcium ions and not to the hypotonicity. These experiments were two in number; in the first of them a strip was exhausted in $\frac{n}{8}$ sodium chloride and then transferred to moist air; while beating feebly in this environment it was moistened first with $\frac{n}{80}$ sodium chloride and afterward with a calcium chloride solution isotonic with it; the effect of moistening with the hypotonic sodium chloride solution was a slight but clearly perceptible increase in vigor which endured for about two minutes; the effect of applying the hypotonic calcium chloride solution was a marked increase in vigor, five times as great as after the use of the sodium chloride solution, and enduring more than ten times as long. Frequently the beneficial effects of a hypotonic calcium chloride solution are much more marked than in this case.[1] The second experiment was conducted along the lines of those for developing rhythmic contractions without the use of sodium salts; a fresh strip was suspended in moist air and surrounded with carbon dioxide for three minutes; the carbon dioxide was then swept out by a stream of air and four drops of distilled water poured onto the strip; six moderately vigorous contractions were given; in two minutes the strip was again moistened with distilled water, and gave three less vigorous contractions; a third moistening with distilled water was followed by three very feeble contractions; three subsequent applications of distilled water were without result; moistening with $\frac{n}{80}$ sodium chloride also failed to induce response; but when the strip was subsequently moistened with 0.1 per cent calcium chloride, a series of twenty or more very vigorous contractions followed promptly.

A comparison of the series of contractions given by strips under the above-described calcium chloride, carbon dioxide treatment with those which follow immersion in $\frac{n}{8}$ sodium chloride reveals several marked differences which are to be brought into line, if

[1] MARTIN: This journal, 1904, xi, p. 126.

possible, with the general view herein presented. The most conspicuous of these differences are as follows: whereas series given by strips in $\frac{n}{8}$ sodium chloride are invariably characterized by a regular and rather rapid rhythm, which continues, with gradually diminishing vigor, to complete exhaustion, those given by strips under carbon dioxide, calcium chloride treatment are apt to show marked irregularities of rhythm, varying from a very slow rate to one more rapid, but in the writer's experiments always less than that which is usual in strips immersed in saline; instead of a continuous decline in vigor, such as is characteristic of sodium chloride series, there was in the strips in air a gradual increase in extent of contraction during the early part of the experiment, such that the most vigorous beats given under this treatment considerably exceeded the best efforts of strips in saline; finally there seemed to be present in the strips under carbon dioxide, calcium chloride treatment a constant inhibitory influence which manifested itself frequently by abrupt cessations of activity for several minutes at a time, and sometimes brought the series to a sudden and unexpected end; this peculiarity is very marked when one compares it with the persistence with which strips in sodium chloride continue to beat till completely exhausted. These differences are to be explained, in the author's opinion, chiefly through the physical differences involved in the two methods of treatment; a strip immersed in a salt solution has the advantage of an environment more nearly normal than that of one in air; in it also, according to the author's hypothesis, there is an intramolecular conversion of calcium from the ineffective to the effective form, which resembles the normal course of events much more nearly than does the introduction of diffusible calcium ions from outside; there is also opportunity, in the strip immersed in saline, for diffusion changes such as cannot occur in one suspended in air. These advantages are sufficient to account for the superiority exhibited by sodium chloride series with respect to regularity of rhythm, rapidity of rate, and continuousness of activity. The features in which strips under carbon dioxide, calcium chloride treatment excel those in saline are greater extent of contraction and the frequent absence of that continuous decline in vigor which is so characteristic of sodium chloride series. The author has elsewhere[1] stated his adherence to the view of Howell[2] that the decline in sodium chloride solution is due to loss

[1] MARTIN: This journal, 1906, xv, p. 315.

[2] HOWELL: *Ibid.*, 1901, vi, p. 203.

of calcium by diffusion, and if that idea is correct then there is no reason to expect a strip in air to show a similar phenomenon. The writer believes that the greater vigor shown by strips in air is probably due to a more active oxidation under those circumstances than is possible in a strip immersed in a sodium chloride solution even under most favorable conditions. It should be stated that the extent of contraction reached its maximum after the first stages of the experiment, and after the treatment with carbon dioxide had been discontinued, a fact which points to the relationship which the author has already suggested between activity of oxidation and extent of contraction.[1]

In the preceding paragraphs the writer has stated his views as to the part played by calcium and sodium ions in ventricular tissue. It remains to consider briefly the influence exerted by potassium ions in the same tissue. There seems to be no question of the inhibitory nature of this influence; the permanent standstill of isolated ventricular tissue immersed in Ringer's mixture can scarcely be explained on any other hypothesis, but, as the author showed above, the potassium ions normally present in ventricular tissue do not exercise an effective inhibitory influence upon it, although their concentration therein is as great as in Ringer's solution. This difference in influence between tissue potassium and inorganic potassium is probably to be accounted for in the way suggested by Howell[2] and the writer,[3] namely, as due to the fact that potassium ions are not actively inhibitory so long as they are in the indiffusible combination in which they occur in tissue, but are so when present in diffusible form, as in Ringer's solution.

The Influence of Salts on the Venous Tissues of the Heart.

It is the belief of the author that the influences exerted by the inorganic blood salts upon this part of the heart are in substantially the same direction as upon the ventricle, and that the differences in their effects upon the two tissues are due to differences in the tissues. The conception that the function of calcium ions is to promote oxidation accords fully with all that is known of the conditions of activity

[1] Martin: *Loc. cit.*, p. 316.
[2] Howell: This journal, 1906, xv, p. 291.
[3] Martin: *Ibid.*, 1904, xi, p. 137.

in venous heart tissue, but since the author has taken the position that automatic rhythmicity depends, indirectly at least, upon free oxidation, and so upon diffusible calcium ions, and since venous tissue possesses this property to so much greater degree than does the ventricle, a difference must be assumed in the relationship of the calcium to the two tissues. A suggestion that will account for the much higher degree of rhythmicity in venous tissue, as compared with ventricular, is that there is a fundamental difference in the calcium compounds in the two tissues such that whereas, in the latter, the calcium normally occurs in an indiffusible, inactive combination, in the former no compound in which calcium is indiffusible is present, but the calcium ions are always in condition to carry on their function of promoting oxidation.

The rôle which was assumed for sodium ions in ventricular tissue, to change by their mass action the form of the calcium tissue combination, is of course a purely extraneous function, one that would never come into play during the normal life of the organ, inasmuch as occasion for the development of spontaneous ventricular activity never arises, so that thus far in this paper there has been proposed no normal rôle for the sodium content of the tissue. If the author's idea, that in the venous tissues the calcium is normally always present in active form, be correct, he cannot see that there is any necessity of assuming for sodium ions a stimulating function in connection with the rhythmic activity of the heart; he would be content to return, in the main, to Howell's[1] old view that the sodium salts function chiefly in the maintenance of favorable conditions for activity, osmotic and otherwise, rather than in active promotion of contraction processes.

A striking difference between venous and ventricular tissues is the greater resisting power of the former to the inhibitory influence of inorganic potassium-containing solutions; it may continue active under the influence of a solution containing potassium ions in six times the concentration necessary to hold ventricular tissue in complete quiescence.[2] The reason for this difference cannot be satisfactorily accounted for until the mechanism of potassium inhibition is understood, and as yet no very definite suggestions have been offered as to the nature of this mechanism. In a future paragraph the author purposes to consider it more in detail.

[1] Howell: This journal, 1898, ii, p. 80.

[2] Martin: *Ibid.*, 1904, xi, p. 386.

The Influence of Salts on Skeletal Muscle.

Attempts to compare skeletal and cardiac muscle with respect to the influence of the inorganic blood salts upon them are at once engaging and baffling: engaging because of the many suggestive points of similarity which they exhibit, and baffling on account of the perplexing differences of reaction which confront the worker who tries to formulate a satisfactory comparative view of the relations of salts to tissues. Some observers have taken the ground that what appears to be the most deep-seated difference between the two sorts of muscle, namely the presence of automaticity in the one and its absence in the other, is really not a fundamental structural difference at all, but only a superficial one, depending upon environmental differences in the condition of the tissues, and probably disappearing if identical conditions could be obtained. The fibrillary twitches which occur in skeletal muscle immersed in alkaline sodium chloride solution are by these observers homologized with the spontaneous rhythmic contractions of heart muscle. It seems to the author, however, that the safest position to take at present is that the twitches given by skeletal muscles are probably not strictly analogous to automatic heart beats, and that the absence of automaticity in skeletal muscle is probably a deep-seated structural characteristic, which is not to be readily overcome by simple changes in the salt content of the tissue. In terms of his hypothesis it may be supposed to be due to the fact that the products of the metabolic activity of this tissue do not function as efficient stimuli in the way in which they do in heart tissue, either because the metabolic products are different or, more probably, because the tissue itself is so constituted as to be unresponsive to that sort of stimulation. Admitting this fundamental difference between the two tissues, and adopting the view that it rests upon differences in reaction between the tissues and their metabolic products, it can be shown that so far as their responses to the inorganic blood salts are concerned, cardiac and skeletal muscle appear very similar. Leaving out of account for the moment the fibrillary twitches given by skeletal muscle in alkaline sodium chloride, and the inhibition of these by calcium chloride, many of the reactions of this tissue to salts, as described by Guenther [1] in his exhaustive study of the subject, can be accounted for very satisfactorily in terms of

[1] Guenther: This journal, 1905, xiv, p. 73.

the author's hypothesis. Thus Guenther shows that in frogs' sartorii immersed in $\frac{n}{8}$ sodium chloride there is a rapid diminution in the vigor of response to electrical stimuli, while the addition of a small percentage of calcium chloride to the solution restores the response completely. These phenomena accord with the author's suggestion that the extent of contraction depends upon the amount of oxidation, and that the latter in turn depends upon the presence of diffusible calcium ions, which are lost from the tissue during its immersion in saline, and restored to it by the addition of the calcium chloride. Guenther's remaining observations concern themselves with those slow variations in length which we call tone changes, and which are not strictly comparable with muscular contractions of the ordinary type, it being generally held that they involve a different part of the muscle substance.[1] Although this paper concerns itself with the functions of the inorganic blood salts in respect to ordinary contraction phenomena in muscular tissues, and although the writer is of the opinion that there is no reason to suppose that their functions in that respect necessarily bear any relation to their influence upon tone changes, yet certain of Guenther's observations upon tone changes are in line with the author's hypothesis and will be referred to in this connection. That author shows, for example, that in sartorius muscle, as in a ventricle strip, treatment with calcium chloride is likely to result in a condition of excessive tone — to be explained in both cases, according to the writer's view, as being the result of over-oxidation brought on by the presence of calcium chloride in too great concentration. The same observer calls attention also to the fact that the contractions produced in skeletal muscle by strong solutions of potassium chloride occur only when calcium ions are present in the tissue, a fact in agreement with the author's suggestion as to the rôle of calcium.

The fibrillary twitches given by skeletal muscle in saline appear to be ordinary muscular contractions of small extent; they are executed rapidly and, as Guenther[2] points out, may involve the whole muscle, even though only a part of it be immersed, differing markedly in this respect from tone changes, which involve only that part of the muscle actually in contact with the bath. Since they occur in a tissue in which such contractions usually appear only as the result of the appli-

[1] Fano: Beiträge zur Physiologie. Leipzig, 1887; Bottazzi: Journal of physiology, 1897, xxi, p. 1. Quoted from Howell's Text-book of Physiology, 1906.

[2] Guenther: *Loc. cit.*, p. 95.

cation of stimuli, it would seem that the saline bath should be compared with other irritants of skeletal muscle rather than be at once assumed to affect this tissue in precisely the same way as it does heart tissue, whose conditions of activity are known to be widely different. Skeletal muscle tissue which is drying from exposure to air gives twitches very similar to those which occur in saline, and these are generally conceded to be irritation effects pure and simple. The inhibition of sodium chloride twitches by calcium ions is to be considered a direct counteraction of the irritating influence of the saline, because, as quoted above, calcium ions are favorable to the response of the tissue when it is stimulated by other agents, electric shocks for example. If the writer's suggestion of the function of calcium ions be correct it would seem likely that the irritation manifested in sodium chloride solution is really some sort of a suffocation effect, which is abolished through the improved oxidation resulting from the application of the calcium ions. This idea is to be considered only a tentative one, there being no direct experimental evidence either in favor of or against it.

It should be kept clearly in mind that, according to the author's hypothesis, improved oxidation under the influence of calcium ions has very different manifestations in heart muscle and in skeletal muscle. In the former the augmented metabolic processes lead to the production of an "inner stimulus" which may cause the tissue to execute rhythmic contractions; in the latter the metabolic activity of the tissue may be also augmented, but since the products which are formed have no stimulating function, there arises no tendency to the occurrence of automatic contractions, although at the same time the ability of the tissue to respond to stimulation is markedly increased.

The Function of Calcium Ions.

The author has assumed for calcium ions a specific function in connection with the metabolic activities of muscle. In this paper he has described this function as that of improving the reactions of the tissue with oxygen. In a former paper[1] the less definite expression, "improving the absorption of oxygen" was used. Inasmuch as there is at present no single generally accepted view as to the mechanism of physiological oxidation, nor as to the exact part played by atmospheric oxygen in the process, it is of course premature to attempt to particularize with definiteness in regard to the manner in which the

[1] Martin: This journal, 1906, xv, p. 303.

different constituents of the tissue may influence it. The author may be permitted, however, to suggest a possibility which has occurred to him to the effect that the action of the calcium in promoting physiological oxidations may be of the same sort as its action in connection with the coagulation of the blood. It has been shown that the production of active thrombin depends upon a reaction between thrombogen and calcium; a similar function for calcium in connection with the activation of the enzyms upon which physiological oxidations probably depend is quite conceivable. Such a conception accords with all that is known of the relations of calcium to normal muscular activity, as the author has been at pains to show, and at the same time is in entire harmony with present views relating to the formation of enzyms in the animal body. In a former paper[1] the author developed the conception that activity in calcium ions, and for that matter in ions generally, depends upon their presence in diffusible form, and that their normal occurrence in animal tissues is in some indiffusible form. This idea has received support from several observers,[2] and seems to be sufficiently well grounded to be accepted provisionally. In it is to be found a suggestion as to the mechanism by which oxidation processes may be controlled, it being possible that the conversion of calcium from one form to the other is subject to both nervous and chemical influences, through which the coordination of the extent of oxidation with the needs of the tissue is accomplished.

The Function of Sodium Ions.

There is one fact in connection with the influence of sodium salts upon ventricular strips which has not, in the writer's opinion, received sufficient consideration, in view of the extent to which it enters into experiments. That is the fact that sodium salts, to be effective in arousing this tissue to activity, must always be applied in quantity; all observers who have worked with sodium-containing solutions have noted this necessity; the author himself has made numerous attempts to obtain, with small quantities of sodium chloride, results such as can readily be had with small quantities of calcium chloride, but his efforts have proved wholly fruitless, so that he has become convinced that the influence of sodium chloride is mainly, if

[1] Martin: This journal, 1904, xi, p. 133.

[2] Stiles and Beers: This journal, 1905, xiv, p. 133; Guenther: *Loc. cit.*, p. 93; Howell: This journal, 1906, xv, p. 294.

not wholly, a mass action influence — that its ions do not themselves exert any very positive tendency toward the incitement of contractions, but that they may replace other ions by their mass action and that the sodium-tissue compounds thus formed are favorable to the carrying on of the vital processes, which are initiated by the deposed ions or by other agencies. The author does not undertake to deny that sodium ions may be absolutely essential to life, and hence to all manifestations of vital activity, but he does hold that positive influence in the direction of arousing automatic contractions is not proven for sodium ions, either in the case of the non-automatic ventricle, or of the highly automatic venous heart tissue.

The Function of Potassium Ions.

The phenomena illustrating the influence of potassium ions on heart tissue are well marked and positive enough to leave no doubt as to the fact of its inhibitory nature, but they are not of a sort to offer any very positive suggestions as to the mechanism by which this inhibitory influence is exerted. There is reason to believe, as Howell[1] suggests, that potassium ions are inhibitory only when present in diffusible form, and that as they normally occur in the tissues they are not in a diffusible form and so are not inhibitory. Several of the facts that have been observed in connection with the inhibition brought on by diffusible potassium ions are interesting, and may possibly afford some slight clue as to the manner of its production. In the first place a concentration of potassium chloride (0.04 per cent) which is sufficient to check completely the development of automatic contractions in an isolated ventricular strip, is not sufficient to inhibit contractions aroused by physiological stimuli, either from the auricles or from a connected strip of ventricle in which contractions have been induced by immersion in saline (Guenther);[2] in the second place concentrations of potassium chloride, which inhibit completely the response to physiological stimuli (0.1 to 0.5 per cent), may not suffice to prevent contractions when mechanical stimuli are applied;[3] in the third place several times as great concentration of potassium chloride is necessary to inhibit the auricle as to inhibit the ventricle; in the fourth place inhibition only continues as long as the diffusible

[1] Howell: This journal, 1906, xv, p. 294.
[2] Guenther: *Loc. cit.*, p. 96.
[3] Martin: This journal, 1904, xi, p. 374.

potassium ions are actually present in the tissue; they may be washed out quickly and such washing out is followed promptly by a resumption of activity,[1] provided the tissue is in an active condition; finally, many observers have shown that there is antagonism of function between potassium and calcium.

According to the author's hypothesis potassium ions might exert their inhibitory influence, either indirectly by interfering with that action of calcium ions upon which proper oxidation depends, or directly by hindering the contraction process itself through the formation of non-contractile compounds, or in some similar manner. In view of the facts cited above, it seems to the writer altogether likely that the potassium ions tend to inhibit both the action of the calcium ions and the contraction processes themselves. A suggestion as to the mechanism of this inhibition can be had if it be assumed that the diffusible potassium ions enter into inhibitory combination with the tissue substances by virtue of their mass action, and that they combine with the zymogen substances which, when activated by calcium ions, become effective in causing oxidation, more readily than they do with those substances which are involved directly in the contraction process. According to this assumption automatic contractions are not developed in a fresh strip of ventricle immersed in Ringer's solution, because the potassium ions present are in sufficient concentration to combine by their mass action with the zymogen substances of the tissue, forming an inactive compound, and preventing the calcium ions, which are also present, from combining with the zymogen to form active enzyms; in this way the conditions of rhythmicity fail to be fulfilled. When a strip under these conditions is stimulated directly, one of the results of the stimulation is to convert some of the calcium tissue compound into a form in which the calcium is active, and so for the moment diffusible calcium ions are present in excess, and there having been applied also a direct stimulus to contraction, the tissue is able to give its response. It is well known that automatic contractions will be given by a strip in Ringer's solution, if the concentration of the calcium salt be slightly increased, and that these may be inhibited in turn by adding more of the potassium salt, and so on, indicating that one or the other enters into tissue combination according as it is in excess. The explanation of the fact that physiological stimulation is inhibited by a smaller concentration of potassium chloride than is artificial stimulation

[1] MARTIN: *Loc. cit.*, p. 377.

would be that the amount of conversion of the calcium tissue compound is greater under artificial than under physiological stimulation, and so a greater number of potassium ions must be present in the former case if activation of the oxidative enzyms is to be prevented. The greater resistance of auricular tissue to potassium inhibition would be explained on the basis of the assumption that in that tissue calcium ions are always present in diffusible form, by supposing that the active oxidative enzymes, which occur normally therein, are not so readily decomposable by potassium ions as are those of ventricular tissue, and so require a greater concentration of the potassium salt to render them inactive.

Howell[1] has advanced experimental evidence in favor of the view that the way in which stimulation of the vagus inhibits the heart is through a sudden conversion of potassium ions from an indiffusible combination to a diffusible inhibitory one. This hypothesis, in connection with the author's suggestion as to the mechanism of the interaction between potassium and calcium ions, offers an explanation for an interesting observation of Loewi and Ishizaka.[2] These authors observed that muscarinized frogs' ventricles retain their irritability if an inorganic calcium salt is present about the tissue but not otherwise. According to the generally accepted view of the mechanism of muscarin action this would be interpreted to mean that the stimulation of the cardiac vagus endings by the drug did not induce sufficient increase in the number of diffusible potassium ions to overbalance the diffusible calcium ions, which are present in considerable numbers under the conditions of the experiment. If the position be taken that muscarin acts to paralyze the muscle substance itself, the interpretation of Loewi's and Ishizaka's phenomenon would be that the paralysis involves only the calcium tissue compound, thus preventing normal activity by hindering the conversion of calcium to the active form, but in no way interfering with the ability of the tissue to make use of inorganic calcium ions when such are supplied, and so to continue irritable.

Summary.

1. Consideration of the possible sources of the "inner stimulus" for the rhythmic activity of the venous heart tissues leads to the conclusion that Howell's original assumption, assigning to certain of the

[1] Howell: This journal, 1906, xv, p. 291.

[2] Loewi and Ishizaka: Centralblatt für Physiologie, 1905, xix, p. 595.

inorganic blood salts the function of arousing it, has not sufficient experimental support to justify its adoption in the face of Langendorff's contention that his assumption, which makes the products of the tissue's own metabolic activity the source of stimulation, is logically more probable. The previously unreported fact is brought out that the duration of vigorous activity in venous strips immersed in normal Ringer's solution is many times longer when the solution is kept saturated with air than when it is not so saturated.

2. In connection with the question as to the nature of the inner stimulus for the ventricle, it is shown that a sodium chloride solution saturated with carbon dioxide stimulates freshly isolated strips of ventricle to immediate activity, differing in that respect from sodium chloride solution or sodium calcium mixtures, in which there is always a distinct latent period. This is interpreted as supporting the view that the "inner stimulus" for the ventricle is furnished by its metabolic products, one of which may be carbon dioxide, and as indicating that calcium ions have an indirect function rather than a directly stimulating one.

3. In order to assign rôles to the blood salts, in connection with the activity of the ventricle, the hypothesis is advanced that reactions between the tissue substance and oxygen can only take place in the presence of diffusible calcium ions. The chief function of sodium ions is assumed to be that of causing by their mass action conversion of calcium tissue compounds from their normal form, in which the calcium is indiffusible, to one in which it is diffusible. It is shown in support of this assumption that by proper procedure, which involves the application of diffusible calcium ions, ventricular strips may be roused to rhythmic activity without the use of any sodium salt whatever. The view that potassium ions are inhibitory only when present in diffusible combination is supported, and it is argued that in their normal tissue combinations they are indiffusible and so are not inhibitory.

4. The high degree of rhythmicity exhibited by venous heart tissue is assumed to be due to the fact that in it calcium does not form an indiffusible tissue compound, hence is always available for use. Sodium ions are assumed to have the function in this tissue of maintaining favorable conditions for activity without themselves entering markedly into the contraction processes.

5. The position is taken that the non-automaticity of skeletal muscle is a fundamental characteristic, and one to be taken into

account when it is compared with the highly automatic heart muscle. It is shown that calcium ions favor the response of skeletal muscle to normal stimulation, just as they favor that of heart muscle to its "inner stimulus." The fibrillary twitches given by skeletal muscle in sodium chloride solution are assumed to be due to an irritation which is removed when a calcium salt is added.

6. It is suggested that the action of diffusible calcium ions in promoting oxidation processes may be in the nature of the activation of enzyms.

7. The assumption that sodium ions exert their influence through mass action is shown to be supported by the fact that sodium salts are effective only when present in quantity.

8. It is suggested that diffusible potassium ions cause inhibition by forming inactive compounds with the zymogens of the tissue and with the contractile substances as well, thus interfering both with the oxidative reactions and with the process of contraction itself.

ON THE CAUSE OF THE CESSATION OF THE RHYTHM OF AUTOMATIC TISSUES IN ISOTONIC SOLUTIONS OF NON-ELECTROLYTES.

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I.

IN the Limulus heart the myogenic theory is eliminated by direct and decisive experiments, but while Volkmann's theory of the mechanism of the heart beat is no longer a theory but a demonstrated fact for that animal, the nature of the stimulus to the heart rhythm is just as obscure as before. We know that the heart muscle contracts in response to rhythmical nervous impulses reaching it from the ganglion. The heart muscle does not, therefore, need any further attention. The next step in the elucidation of the nature of the heart rhythm is to determine the nature of the stimulus to the ganglionic rhythm. This paper reports some observations bearing on the question whether the immediate stimulus to the automatic heart tissue is to be sought in some constituents of the blood surrounding the tissue or in some substance or process within the cells of the automatic tissue themselves.

The numerous researches of the last thirty years touching this question have been directed mainly towards the inorganic constituents of the blood. Kronecker and his pupils have emphasized the importance of the serum proteids for the heart rhythm, while others have maintained that the presence of sugar in the circulating medium renders the medium more efficient for maintaining the rhythm than an isotonic solution of the inorganic salts of the blood. But while it is obvious that the organic constituents of the serum are necessary for the nutrition of the heart tissues and therefore ultimately for the heart rhythm, the fact that the heart may continue in rhythm for very long periods in isotonic solutions of electrolytes containing no proteids or carbohydrates seems to show that the organic constitu-

ents of the serum do not form the immediate stimulus to the heart. It must be admitted, however, that this view is by no means proved. The slowly diffusing serum proteids probably require hours or even days to pass from the intercellular spaces into the solution surrounding the tissues.

The view that the inorganic constituents of the blood, and particularly the ionized portion of them, constitute the immediate stimulus for the heart rhythm rests mainly on these two facts: 1. The heart, particularly of cold-blooded animals, continues to beat for many hours in an isotonic solution of the ash of the blood, while it is rapidly brought to a standstill in an isotonic solution of a non-electrolyte such as cane sugar. 2. Some of the inorganic constituents of the blood (*e. g.*, Na Cl) produce rhythmic activity in tissues not normally rhythmical (ventricular apex, skeletal muscle, motor cells of the spinal cord). In this country this view is particularly championed by Howell[1] and Loeb[2] and their students. The fact that the parts of the heart normally automatic continue in activity for some time in an isotonic solution of a non-electrolyte, such as cane sugar, is explained by the adherents of the above view in the following way: The rhythm of the heart immersed in an isotonic solution of cane sugar is maintained by the electrolytes in the blood of the capillary spaces between the heart cells, and the cessation of the rhythm marks the point where these electrolytes have diffused from these intercellular spaces into the sugar solution to such an extent that their concentration about the cells is insufficient for their stimulating action. This explanation seems to be further supported by the fact that a heart brought to a standstill in an isotonic solution of cane sugar will resume its rhythm on being transferred to an isotonic solution of the ash of the blood. If this explanation of the cause of the cessation of the rhythm in a solution of non-electrolytes is correct it follows that the duration of the maintenance of the rhythm in the sugar solution is a function of the rate of diffusion of the ions out of the intercellular capillary spaces and independent of the condition of the cells themselves.

While the above view commends itself because of its simplicity,

[1] The normal rhythm of the cold-blooded heart depends upon the fact that *in blood and lymph the proportion of calcium and potassium compounds is such as to cause rhythmic activity of the muscular tissue* at the venous, but not at the arterial end of the heart. Howell: This journal, 1898, ii, p. 81.

[2] Loeb: Festschrift für Fick, 1899, p. 101.

the facts are capable of quite a different interpretation. On the view that the inorganic salts of the blood form only the condition for the heart activity and that the immediate stimulus to the rhythm is some substance or process within the cells themselves, the standstill of the heart in a sugar solution may be accounted for in the following way: The sugar (urea or glycerine) molecules because of their concentration may enter the cells and thus alter the equilibrium within the cells. The diminished pressure of the electrolytes surrounding the cells may cause outward diffusion of some of the electrolytes within the cells. This would again alter the conditions within the cells and perhaps alter their permeability. If this explanation is the correct one, it follows that the faster a particular non-electrolyte enters the cells or alters their permeability in the direction inimical to the rhythm the quicker the heart will come to a standstill in a pure solution of that chemical, independent of the rate of diffusion of the electrolytes out from the intercellular spaces. It follows, further, that in an isotonic solution of a non-electrolyte the duration of maintenance of the rhythm would not necessarily be the same for all hearts of equal size of the same species, but would vary with the conditions of permeability of the automatic cells. The following observations on the activity of the automatic heart ganglion of Limulus in isotonic solutions of non-electrolytes appear to me to support this explanation.

II.

In these experiments the heart ganglion was isolated in the posterior region of the heart, and left in connection with the heart muscle of the anterior end. This preparation was arranged for graphic registration in the manner described in a previous paper.[1] The part of the heart muscle used for contraction, and thus for recording the activity of the ganglion, was surrounded by plasma or sea water, the solutions of non-electrolytes being confined to the ganglion.

The blood of Limulus is isotonic with the sea water. The osmotic pressure of the Limulus blood is therefore nearly the same as that of a gram-molecular solution of cane sugar, the Limulus plasma depressing the freezing point $\triangle - 1.82°$ C.,[2] a molecular solution of cane sugar depressing the freezing point $\triangle - 1.87°$ C.

[1] CARLSON: This journal, 1906, xv, p. 207.

[2] GARREY: Biological bulletin, 1905, viii, p. 257.

When the plasma or sea water surrounding the ganglion is replaced by an isotonic cane sugar solution, the rhythm is nearly always augmented in rate and usually also in intensity. This stimulating action may appear within ten to sixty seconds after the application of the sugar solution. The stimulating action is followed by irregularity in the rhythm, diminution in the intensity of the nervous discharges, diminished rapidity of the nervous discharges, and finally complete cessation of the rhythm. The primary stimulating action of the sugar solution was not due to impurities in the sugar; nor was it due to differences in temperature of the plasma and the sugar solutions replacing the plasma, as particular care was taken to keep the solutions employed at the same temperature. This primary stimulating action is not peculiar to cane sugar. Isotonic solutions of dextrose, lævulose, and lactose gave practically the same results. In fact, these sugars appear to have the same action on the isolated ganglion. The fact that the sugars stimulate the ganglion in isotonic concentrations will be considered more fully in connection with the activity of this ganglion in solutions of electrolytes.

The Limulus heart ganglion in adult specimens is about 1 mm. wide at its widest portion, and only a fraction of a mm. thick. This small piece of tissue can therefore hold only a very small amount of plasma in the intercellular spaces. Diffusion outward into the sugar solution of the salts contained in the tissue spaces at the moment of transfer of the ganglion from the plasma to the sugar solution is not hindered by any membrane surrounding the ganglion. The amount of sugar solution used for the bath did not seem to affect the duration of the rhythm in the sugar solution. A ganglion placed in 200 c.c. of the sugar would come to a standstill as quickly as one bathed in 20 c.c. of it.

The length of time a ganglion continues in rhythmic activity in an isotonic solution of pure cane sugar depends on the condition of the ganglion. This can be shown in two ways. A ganglion in good condition, that is, a ganglion not injured in the dissection and that exhibits a regular and vigorous rhythm, as judged by the contraction of the heart muscle, may continue in activity in an isotonic solution of pure sugar for sixty to ninety minutes. That is the longest time I have observed any ganglion in activity in the sugar bath. The usual time for ganglia in good condition is from forty-five to sixty minutes. A ganglion in "poor" condition, that is, a ganglion exhibiting a relatively feeble, slow, and irregular rhythm at the beginning

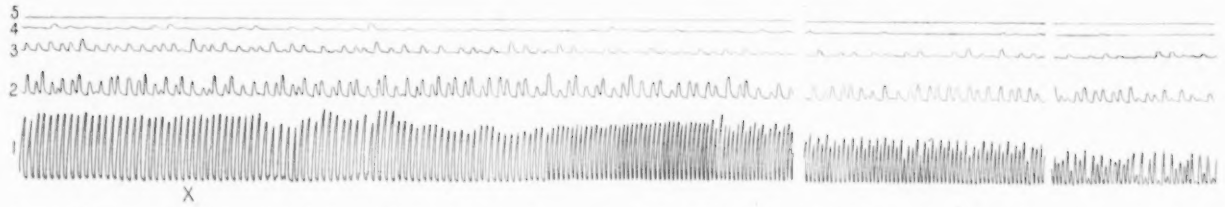

FIGURE 1. — Two-thirds the original size. Tracing from the first segment of the Limulus heart left in connection with the ganglion isolated from the heart muscle posteriorly. The records are the successive tracings; the left hand corner of the lower tracing is separated by an interval of sixty-five minutes from the right end of the upper or last tracing. *X* = the isolated ganglion immersed in an isotonic solution of cane sugar (200 c.c.). Showing primary stimulation, irregularity, depression, and final cessation of the ganglionic rhythm. The beats are still discernible on the upper (5th) record by the aid of a hand lens.

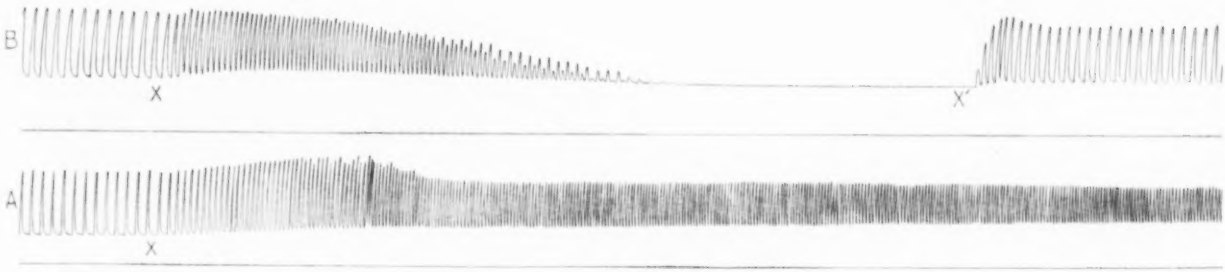

FIGURE 2. — Two-thirds the original size. Tracings from the anterior end of the Limulus heart left in connection with the nerve cord isolated posteriorly. *A*, beginning of the record of the first bath of the ganglion in $\frac{m}{10}$ molecular solution of lævulose. *X*, application of the lævulose solution to the ganglion. *B*, second bath of ganglion in the sugar solution after recovery of the rhythm in plasma. *X*, application of the sugar solution to the ganglion. *X'*, the sugar solution replaced by plasma. Showing the much shorter duration of the ganglionic rhythm in the second sugar bath as compared to the first.

of an experiment is brought to a standstill in the sugar solution in from eight to twenty minutes. This difference in the duration of the rhythm of the vigorous and the poor ganglion in the same sugar solution is thus very marked. The fact was recorded both on the tracings and in the protocols before I saw its bearing on the question now under consideration.

Why does the vigorous ganglion maintain its rhythm in the sugar solution so much longer than the feeble ganglion? The feeble ganglion is of the same size, texture, and compactness as the vigorous ganglion. The amount of plasma imprisoned in the intercellular spaces must be practically the same in both. The rate of outward diffusion of the salts in this imprisoned plasma must be the same in both. If, therefore, the cessation of the ganglionic rhythm marked the time when the outward diffusion of the electrolytes surrounding the cells had diminished the electrolytes about the cells down to certain concentration below which they fail to stimulate the ganglion cells, the vigorous ganglion should come to a standstill as soon as the feeble ganglion. It seems to me, therefore, that the fact just recorded is not adequately explained on the view that the cause of the standstill of rhythmical tissues in solutions of non-electrolytes is the absence of or dilution of the electrolytes about the automatic cells.

III.

A ganglion brought to a standstill in an isotonic sugar solution resumes its rhythmic activity when again surrounded by plasma or sea water. The return of the rhythm is gradual, but the restored rhythm may for a while exceed the original both in rate and intensity. If the ganglion is transferred to the plasma soon after coming to a standstill in the sugar solution, the rhythm may reappear within a few seconds and the restored rhythm may continue nearly as regular and vigorous as the original. Nevertheless, *a vigorous ganglion brought to a standstill in the sugar solution and again restored by plasma or sea water will, on a second bath in the sugar solution, come to a standstill much sooner than at the first time.* For example, a ganglion brought to a standstill in forty-five minutes when placed in the sugar solution for the first time, will, after what appears to be complete recovery in plasma or sea water, cease its activity in from ten to fifteen minutes when again surrounded by the sugar. Having once been brought to a standstill in the sugar solution, the ganglion reacts to

the second and third sugar baths as a ganglion in "poor" condition. Here, again, the theory that the standstill in the sugar solution is due to outward diffusion and dilution of the electrolytes about the cells fails to explain the facts. Granted that the electrolytes surrounding the cells constitute the immediate stimulus to the rhythm, there must be as many of them, qualitatively and quantitatively, surrounding the ganglion cells during the restored rhythm as during the original, for the restored rhythm may be as rapid and vigorous as the rhythm preceding the sugar bath. On this view the only explanation of the briefer period of activity in the second sugar bath would be a more rapid diffusion of the electrolytes out from the intercellular spaces, a supposition which has no basis of support in physical chemistry.

Isotonic solutions of other non-electrolytes, such as urea and glycerine, bring the Limulus heart ganglion to an ultimate standstill after a primary stimulation, and the rhythm is restored by plasma or sea water. An isotonic solution of urea causes great primary stimulation of the ganglion, the rate of the rhythm being particularly augmented. Then follows irregularity, depression, and complete cessation within two to three minutes. An isotonic solution of glycerine brings a vigorous ganglion to a standstill in from five to ten minutes. The primary stimulating action of isotonic solutions of urea and glycerine will be considered more fully in another paper. The action of these substances is referred to in this connection merely to emphasize the point that *the action of the non-electrolyte on the automatic tissue is a factor in the cause of the cessation of an automatic tissue in a non-electrolyte.* A vigorous ganglion will continue in activity for about forty-five to sixty minutes in the sugar solution, for about five to ten minutes in the solution of glycerine, and for about three minutes in an isotonic solution of urea. On the view that the cause of the standstill in a solution of a non-electrolyte is the dilution of the electrolytes about the cells owing to diffusion outward, the more rapid cessation of the rhythm in the urea and the glycerine solutions can only be due to a more rapid diffusion of the salts in the intercellular spaces than is the case in the ganglion placed in the sugar solution, a supposition which has no basis in physical chemistry. And if the theory is inadequate with reference to cessation of automatic tissues in solutions of urea and glycerine — if we are forced to take into account the action of these substances on the cells themselves — what reason is there to assume that the same factor does not play a part in the cessation of automatic tissues in

sugar solutions? That the urea and the glycerine act directly on the ganglion cells appears to me to be shown by the feeble and irregular rhythm of the ganglia after a urea or glycerine standstill. The action of the urea is the more deleterious. That the sugar molecules act directly on the ganglion is indicated by the fact that the second sugar bath brings the ganglion to a standstill within a shorter time. Concerning the probable nature of this direct action of these non-electrolytes on the ganglion cells in bringing about cessation of the rhythm nothing definite can be said at present.

There are many points of similarity between the chemical conditions of the automatism of cilia of marine animals and the chemical conditions for the activity of the heart. It has been observed that cilia may continue in activity after being severed from the cells. The immediate stimulus to the ciliary automatism must then be sought either within the cilium itself or in the medium surrounding the cilium. Now, as a matter of fact, the cilia of marine animals are quickly brought to a standstill when surrounded by an isotonic sugar solution,[1] just as the heart is quickly brought to a standstill in a similar solution. This ultimate cessation of the ciliary contractions in a non-electrolyte may be ascribed to the absence of the stimulating ions in the medium surrounding the cilia with just as much reason as the same explanation is advanced for the standstill of the heart. The cilia of fresh water animals react quite differently in isotonic solutions of sugar. The cilia of Paramœcium, for example, continue in activity indefinitely in distilled water or isotonic cane sugar solutions.[2] In such cases the immediate stimulus to the ciliary rhythm cannot be sought in the surrounding medium. While it is possible that the immediate stimulus to ciliary contraction is different in marine and fresh water animals, it does not seem probable. The difference in the behavior of marine and fresh water cilia in distilled water and isotonic solutions of non-electrolytes is probably due to differences in permeability to the substances in solution within the cilia as well as to the water and salts surrounding them.

Summary.

1. The duration of the maintenance of the activity of automatic tissues in isotonic solutions of non-electrolytes (sugar, urea, glyc-

[1] Lillie: This journal, 1902, vii, p. 50.
[2] Towle: This journal, 1905, xii, p. 220.

erine) depends on the condition of the automatic tissues and the specific nature of the non-electrolyte, and not directly on the rate of diffusion of the electrolytes of the plasma in the intercellular spaces into the solution of the non-electrolyte. It is, therefore, in part due to some action of the non-electrolyte on the automatic tissue.

2. The cessation of the rhythm of the automatic heart tissue in solutions of non-electrolytes can therefore not be used as an argument in support of the view that the immediate cause of the rhythm of the automatic heart tissue is to be sought in the electrolytes of the plasma surrounding the tissue.

THE PRESENCE OF CARDIO-REGULATIVE NERVES IN THE LAMPREYS.

By A. J. CARLSON.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

SOME years ago Greene made the observation that the California hagfish (Bdellostoma dombeyi) is not provided with cardio-regulative nerves.[1] According to Greene the heart rhythm of this animal cannot be influenced by the stimulation of the vagi, the brain, or the spinal cord. This observation is of peculiar interest, because the hagfish is the only vertebrate animal on record in which cardio-regulative nerves are absent. A little later I had an opportunity to repeat Greene's experiment on the hagfish, and my observation confirmed Greene's conclusion. I was not able to get evidence of any extrinsic heart nerves that influence the heart rhythm in that animal.[2]

It is obvious that a vertebrate heart not provided with accelerator and inhibitory nerves offers peculiar advantages for the investigation of certain questions of heart physiology. With that idea in view I endeavored to determine whether the absence of cardio-regulative nerves was a characteristic of the whole marsipobranch group of animals or limited to the hagfishes alone. The lampreys have a much wider distribution than the hagfish, and are available even for the physiological laboratories situated inland. The only lamprey material that I was able to obtain on the Pacific coast was the larval form of Entosphenus tridentatus, specimens 12 to 14 cm. in length. And as I was unable to demonstrate any influence on the heart of the stimulation of the vagi, the brain, or the spinal cord in these larval representatives of the lampreys, I concluded that the absence of cardio-regulative nerves was in all probability a characteristic of the whole marsipobranch group, that is, both hagfishes and

[1] GREENE: This journal, 1901, vi, p. 318.

[2] CARLSON: Zeitschrift für allgemeine Physiologie, 1904, iv, p. 259.

lampreys. But subsequent observation on two species of adult lampreys have convinced me that this conclusion is erroneous.

The best material on which to determine this point is undoubtedly the large marine lamprey of the Atlantic seacoast, but as I was not able to secure this material the smaller river lampreys had to suffice. The species worked on were Ichthymyzon castaneus and Ichthymyzon concolor.

These lampreys are of small size and the vagi are deeply imbedded in the tissues surrounding the gill pockets and therefore not readily isolated for purposes of physiological experiments. In fact, most of the observations were made by stimulation of the medulla at the level of the origin of the vagi. The exact course of the cardio-regulative nerves from the central nervous system to the heart is therefore left undetermined. But my results show conclusively that the central nervous system is connected with the heart by ordinary augmentor and probably also by inhibitory nerves.

The following procedure is most convenient in order to demonstrate the influence of the central nervous system on the heart rhythm. The animal is transsected just behind the heart, secured to a board ventral side down, and the brain exposed. The preparation is now tilted sidewise, the body well slit open in the ventral median line, the heart exposed by cutting away the posterior part of the cartilaginous pericardium. The heart can now be observed directly. The removal of the posterior part of the cartilage is not absolutely necessary, as the cartilage is sufficiently transparent to observe the movement of the heart. But for accurate observation the removal of the cartilage is desirable.

In a specimen thus prepared the stimulation of the medulla with a weak interrupted current usually inhibits the heart for a short period (five to ten seconds, seldom longer). On cessation of the stimulation the rhythm is augmented. If a relatively strong interrupted current is used augmentation of the rhythm is invariably produced. In many specimens the inhibition was not obtained by the weak current, every strength of the stimulus that affected the heart producing augmentation of the rhythm.

The stimulation of the medulla at the level of the origin of the vagi nerves produces contraction of the gill pockets and the trunk muscles. That the changes in the heart rhythm are not an indirect effect of these contractions is shown in the following way: The contraction of the trunk muscles can be abolished by destruction of the

spinal cord, and after the cord has been destroyed below the medulla the stimulation of the medulla still produces the above described effects on the heart. The contraction of the gill pockets that are innervated by the vagi might influence the heart by increasing the resistance to the flow of blood from the ventricle as well as by forcing blood into the sinus by way of the ventral vein. That the resistance to the output of the ventricle is not a factor is shown by the fact that the same results are obtained when the heart is empty or even after the ventricle is severed from the aorta. Nor do the contractions of the gill region produce the change in the rhythm by mechanical tension or pressure on the heart, because the body wall above the heart may be fixed in a way so that the contraction of the gill region does not produce any pressure or tension on the heart.

The conclusion seems, therefore, justified that these lampreys are provided with cardio-regulative nerves in the same way as are the fishes and higher vertebrates. The nerves reach the heart along the vein that joins the sinus in the ventral median line. This is shown by physiological experiments. As long as this connection with the sinus is intact the medulla exhibits its usual influence on the heart. This corresponds with the descriptions and figures of the cardiac branches of the vagi in Myxine by Müller.[1] In the specimens of lampreys at my disposal these cardiac branches of the vagi are too delicate to be made out by dissection, or at least I failed to do so, but by the methylene blue method I have repeatedly brought out an abundance of nerve fibres (non-medullated) in the walls of this ventral vein or sinus leading to the auricle from the gill region.

That two groups of animals as closely related as the hagfishes and the lampreys should exhibit this great difference in this heart physiology was certainly surprising to me. Perhaps the question should be re-examined in the hagfish.

[1] MÜLLER: Vergleichende Anatomie der Myxinoiden. Berlin, 1834.

PHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES OF MAGNESIUM SALTS. — III. THE NARCOTIZING EFFECT OF MAGNESIUM SALTS UPON NERVE FIBRES.

By S. J. MELTZER and JOHN AUER.

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Introductory.

OF many known anæsthetics and depressants it is safely established that they are capable of narcotizing nerve fibres, *i. e.*, they interrupt conductivity and abolish excitability of nerve trunks when they are applied directly to them. The best known anæsthetics, ether and chloroform, were extensively studied in this regard. Local narcosis of nerves is produced also by carbon monoxide and dioxide, the vapors of ethyl alcohol and ammonia. Volatility is a property common to all the foregoing substances and it might be claimed that it is an important factor in the production of local anæsthesia of nerves. There are, however, also some non-volatile substances of which it was demonstrated that they are capable of abolishing excitability and conductivity of nerves by simple local application; we may mention here: cocaine, phenol, chloral (Wedensky), and perhaps also chloralose (Herzen).

In previous communications on some physiological and pharmacological effects of magnesium salts, we reported results of experiments which we have carried out by means of subcutaneous and intravenous injections of these salts.[1] The striking and most general effect of these injections was the production of anæsthesia, either general anæsthesia alone, or in addition to it, also anæsthesia (inhibition) of the centres of the medulla oblongata. In these experiments the magnesium salts affected mainly the nerve cells of the central nervous system, or, to be more particular, the nerve cells of the

[1] S. J. Meltzer and John Auer: This journal, 1905, xiv, p. 366 and 1906, xv, p. 387.

cerebral cortex and of the centres of the medulla oblongata. There was no evidence in these experiments that nerve fibres may also be affected by magnesium salts. The abolition of excitability of some of the nerves, which was observed in these experiments, like the impossibility to affect the respiration by stimulation of the vagus or to bring out deglutition by stimulating the superior laryngeal nerve, etc., was apparently due, as we have argued in the above mentioned communications, to an inhibition of the nerve cells in which these nerves terminate and not to a narcotization of the nerve fibres themselves. There were, however, no *a priori* reasons against the assumption that magnesium salts might produce a local narcotizing effect upon nerve fibres. And comparing the general anæsthetic effect of the magnesium salts with that of some other anæsthetic substances there is one point which speaks for a greater depressing efficiency in case of the salts in question. While in many of the anæsthetic substances the anæsthesia is not their sole effect, on the contrary the anæsthesia is, as a rule, preceded by a stage of increased irritability, there is no such phase in the effects of magnesium salts. As we have pointed out in the previous papers, we have so far noticed not a single phenomenon among the effects of these salts which could be reasonably interpreted as an irritating influence.

In the present paper we are going to report the results of a series of experiments on the problem under discussion: Whether solutions of magnesium salts have a local anæsthetic effect when applied to nerve trunks.

Method.

Except in a few instances, the local narcotic effect upon nerves of the substances enumerated above was studied upon nerve-muscle preparations of frogs, and frequently after the preparation was kept immersed in an isotonic solution of sodium chloride for many hours. The latter procedure was carried out for the purpose of reducing the irritability of the central part which might have become increased by the effect of the cutting. Such a nerve surely cannot be considered a normal one and might be specially predisposed to become easily narcotized by some anæsthetic agent. Our experiments with magnesium salts were carried out mostly on intact nerves *in situ*, which, therefore, were not deprived of their normal nutrition. Such nerves probably offer greater resistance to the anæsthetization and recover more readily as soon as the anæsthetic agent is removed. The

experiments were carried out exclusively on rabbits. The pneumogastric, cervical sympathetic, depressor, sciatic, and anterior crural nerves were tested. The method of application was simple. A pledget of absorbent cotton was saturated with a solution of magnesium salt and wrapped around a section of the nerve to be tested for a distance of two or three centimetres. Frequently the cotton while around the nerve was repeatedly moistened again with the magnesium solution. The adjacent sections of the nerve were protected with cotton saturated with indifferent solutions. At first, solutions of 0.9 per cent sodium chloride were employed; we soon found that this is not a real indifferent solution to nerve tissue and therefore subsequently employed exclusively Ringer's solution for this and similar purposes. Before the solutions of magnesium salts were applied, the irritability of the nerve was tested with induction currents and a strength was found which brought out an unmistakable reaction. At various intervals after the application the nerve was tested with the same strength: at the site of application, above, and below it. After the presence of a "block" was established, the section of the nerve which was subjected to the influence of the solutions of magnesium salts was now "washed" with Ringer's solution and at intervals tested for the return of conductivity and irritability of that section.

As in the previous series, again solutions of the sulphate and chloride of magnesium were tested in concentrations varying from 25 per cent (nearly $\frac{m}{1}$ for magnesium sulphate) to such hypotonic solutions as 1.7 per cent of magnesium sulphate and 1.3 per cent of magnesium chloride.

During the operation for the isolation of nerves, etc., the rabbits were anæsthetized by ether and in some cases also by small doses of morphine.

The Effect of Magnesium Salts upon Nerve Trunks.

The vagus. — The vagus contains various nerve fibres the stimulation of which brings out well defined and easily recognizable reactions in several functions. We shall mention here only the following: stimulation of the central end causes, 1, a change in the character and rate of respirations; and 2, often also an increase of blood pressure; stimulation of the peripheral end causes, 1, a change in the rate of the heart beats and sometimes only a visible change in their strength; 2, contraction of the œsophagus, and 3, contraction of the cardia.

We have studied the effect of the solutions upon the different nerve fibres of the vagus belonging to the different functions separately as well as simultaneously.

The respiratory nerve fibres. — Electric stimulation of the vagus nerve either at some place of the intact nerve trunk or at the central end of the cut nerve will cause, if the current is sufficiently strong, some change in the respiration; upon this point all investigators agree. There is, however, some disagreement as to the nature of this change; some writers say that the effect is of an inspiratory, others again that it is of an expiratory character. The literature on this subject is quite extensive, and we shall not attempt on this occasion to enter into the details of this long-standing controversy. However, in order to make some of our statements intelligible, a brief statement of some details is indispensable. In the majority of rabbits a stimulation with medium and strong induction currents will produce an inspiratory effect. Studying it upon a respiratory tracing it presents itself as follows: The stronger current brings out an inspiratory standstill; the normal respiratory undulations are interrupted, and a straight line or a line slightly and irregularly interrupted by short respiratory undulations runs more or less near the inspiratory level. Weaker currents do not stop the respirations, but produce an increase in rapidity with a shortening mostly on the expiratory side of the undulations. In some of these animals a very weak stimulus will bring out a standstill of an undoubted expiratory character, or at least a slowing with a shortening of an expiratory character. There are, however, undoubtedly some animals in which every kind of stimulation produces only an expiratory standstill which becomes more pronounced the stronger the stimulus is. These facts we have to bear in mind when we come to interpret the changes in the reactions brought on by stimulations after a certain degree of block in the nerve is established.

The graphic record of the respirations was obtained either from the pleural cavity by means of the pleural cannula, as described in the previous communications, or from a very large bottle which was connected with the trachea of the animal, the bottle being frequently ventilated by means of bellows. In one case the respirations were transmitted by a small rubber bag which was placed between the diaphragm and the liver.

The following is a brief account of the results:

When a fairly strong electrical stimulus was found which was

capable of producing from any part of the vagus nerve a well marked respiratory reaction, a concentrated solution of magnesium salt, for instance 25 per cent of the sulphate, was applied to some part of the nerve. As a rule, sooner or later after that application the same electric current applied peripherally to the treated part of the nerve will either produce no reaction upon the respiration at all, or the reaction will be slight and correspond to the effect of a much weaker stimulus; the irritability of the nerve centrally to the treated part remained

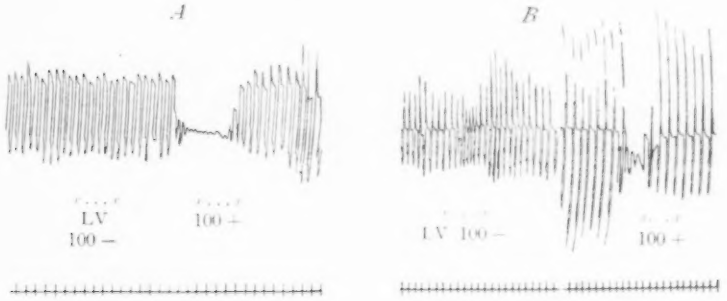

FIGURE 1. — Respiration, inspiration downward, from left to right. L. V. = left vagus. 100 + = stimulated above block with 100 mm. coil distance. 100 − = stimulated below. "*A*" obtained after application of 25 per cent magnesium sulphate. "*B*" obtained after washing with Ringer. Inspiration = down stroke. Time in two second intervals.

unchanged. The solution of magnesium salts greatly reduced or entirely abolished the conductivity of the part of the nerve to which it was applied; it produced a block. In some instances, after bathing the nerve with the solutions, even currents of much greater strength than the original one were not effective, would not pass the block. However, when the cotton would be removed and the nerve left in the wound for some time without being handled, or still better when the nerve was frequently "washed" with Ringer's solution within the blocked part, conductivity sooner or later would return and might even attain its original proportions. (See Fig. 1.) To this we have to add that blocking of the respiratory fibres of the vagus nerve was established not only with strong hypotonic solutions, but was frequently produced also with isotonic solutions (equimolecular with 0.9 per cent sodium chloride), in fact, even with quite low hypotonic solutions, for instance, 1.7 per cent magnesium sul-

phate or 1.3 per cent magnesium chloride. But we may state that the local anæsthetizing effect of the solutions has been as a rule in proportion to their concentration. In the first place, the effect would be profounder with a strong solution; for instance, where a normal molecular solution ($\frac{m}{1}$ — about 25 per cent of magnesium sulphate) would produce a complete block in the section of nerve to which it was applied, an isotonic or hypotonic solution might merely reduce the conductivity in that part. Furthermore, as a rule, after application of strong solutions the effect would set in much sooner than after dilute solutions. Finally, after dilute solutions the return of the normal conductivity would occur more readily and sooner than after the use of high concentrations. In fact, when employing dilute solutions the return of the normal conductivity might take place while the cotton was still around the nerve if no fresh magnesium solutions were added.

All this is true only in a general way. Studying the experiments in detail we find that the effects were variable within comparatively wide limits. In one case, for instance, we find that a 25 per cent solution of magnesium sulphate produced a complete block five minutes after the application, while in another experiment with the same solution it took twenty minutes and more to establish full local anæsthesia. Again, in one instance, the conductivity would return after a short washing of the nerve with Ringer's solution, while in another experiment with the same concentration of magnesium salt and the same duration of application it took perhaps an hour and longer before the return of conductivity and irritability in and below the anæsthetized area. We cannot make any statement as to the exact nature of the cause of this variability. We believe, however, the latter to depend upon accidental, mechanical conditions; for instance, upon the thickness of the nerve, the amount of connective tissue which remains adherent to the nerve, the thin layers of lymph and clotted blood, etc., variable conditions which mechanically hinder the entrance of the solution into the nerve fibres and also hinder its leaving them.

In the foregoing we mentioned essentially the blocking of conductivity of the nerve by solutions of magnesium salts; for we had the positive proof in the fact that stimulation below and outside of the "anæsthetized" area produced no effect. As to the effect upon the irritability within the blocked area our experiments do not permit us to take a positive stand. In the majority of the experiments

stimulation of the anæsthetized area was not without some effect upon the respiration, but it depended upon what part of the anæsthetized area the electrodes were placed. The effect was considerable when the electrodes were near the peripheral border of the blocked area. In two or three instances, however, stimulation of the entire area produced no change in the respiration, while a stimulation below was not without some effect. The last experiences would seem to speak in favor of an assumption that in these cases the irritability was affected more profoundly than the conductivity, a conclusion which would imply at the same time the premise that conductivity and irritability are separate processes. We are, however, of the opinion that our present experiments do not justify our taking a stand in this much debated question, nor do we wish to enter here into a discussion of it.

Some instructive observations were made on the blocking of normal respiratory impulses. As is well known, soon after cutting both vagi in rabbits the respirations become slow. In a number of experiments we have produced the same effect by means of interrupting the normal conductivity through the application of solutions of magnesium salts upon the vagi. In some cases both vagi were intact and the applications were made on both nerves, and in other cases one vagus was cut and the other blocked by the magnesium solution. In all cases some time after the application the respirations began to grow slow, in two cases the respiratory pause became so dangerously protracted that artificial respiration had to be instituted and one animal died (!) in this state. By washing the nerves with Ringer's solution respiration could be restored to its normal state, and in one animal these procedures were carried out twice successfully.

In the series of experiments which we have just mentioned, as well as in other local applications of solutions of magnesium salts, we have never noticed an effect which could be considered as a chemical stimulation of the respiratory fibres. At the moment of the application there was of course sometimes a slight effect, lasting a second or two, which apparently was due to mechanical stimulation by unavoidable handling of the nerves. An increase in the rate of respiration or a slowing of it lasting many seconds, which we could reasonably ascribe to the chemical effect of the solution, was never observed even when using such a strong concentration as 25 per cent of magnesium sulphate.

As we have stated above, the usual effect of a strong electrical stimulation of the central end of the vagus is an inspiratory standstill and the effect of a weaker stimulus is, as a rule, an increase in the rate of respirations with a shortening of the amplitude of an inspiratory character; and that finally in many cases the effect of a mild stimulus consists essentially in an increase of the expiratory pause. In some of our experiments, where the blocking was more or less incomplete, the same stimulus which before the application of the solutions caused, for instance, an inspiratory standstill, would afterward produce only a moderate increase of the rate with some shortening of the amplitude, and in some other cases the later effect would consist only in a slowing of the respirations of an expiratory character. (See Fig. 2 *a*, Figs. 3, 4, and 5.) In many of these cases the very same effects were seen before the application of the solutions, brought out by different strengths of the stimulations.

The facts just mentioned have to be borne in mind when interpreting the results obtained by the different grades of anæsthesia produced by the various dilutions of the employed solutions. Otherwise an inclination may arise to assume that the different degrees of anæsthesia rule out by specific action nerve fibres of a different respiratory character; for instance, that one degree of anæsthesia will affect only expiratory, another only inspiratory, fibres, and still another only sensory fibres. In other words, we would ascribe to the different degrees of anæsthesia specific affinities for the several sets of respiratory fibres, while in reality the uniform effect of the increasing anæsthesia consists merely in reducing the stronger impulses to lesser values. We shall have to discuss this point later more fully. At this place we wish only to call attention to the statement of Howell and his associates in their studies of the effect of cooling upon conductivity and irritability of nerves.[1] Blocking was produced by cooling, and among others the respiratory fibres of the vagus were also tested. Only two experiments were made, one on a cat and one on a rabbit. It was found that by different degrees of cooling of the nerve the same degree of stimulation peripheral to the cooled part produced different effects upon the respiration, and the assumption was made that by the different degrees of cooling, different kinds of fibres (sensory and respiratory) were eliminated. No different degrees of stimulation were tested previous to the cooling. But

[1] W. H. Howell, S. P. Budgett, and E. Leonard: Journal of physiology, 1894, xvi, p. 298.

graphic figures similar to those obtained by the authors after various degrees of cooling, we have frequently obtained from normal nerves simply by using different degrees of current strength in stimulation. We are rather inclined to interpret the results obtained by these authors by the assumption that with the increased cooling the conductivity of the nerves decreased, which in proportion reduced the strength of the stimuli to lesser values; hence the different effects.

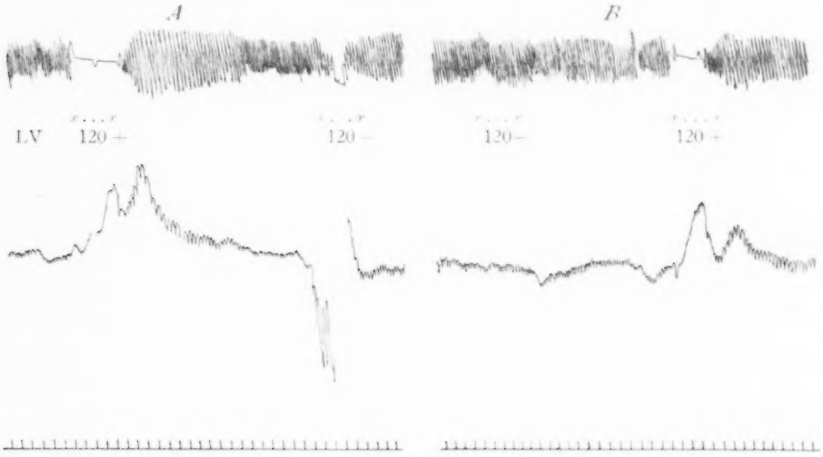

FIGURE 2. — Upper tracing respiration, inspiration upstroke; lower tracing blood pressure. L. V. = left vagus. 120 + = stimulated above block with 120 mm. 120 − = stimulated below. "*A*" obtained ten minutes after application of 25 per cent magnesium sulphate. "*B*" after a second application and ligation of vagus between block and heart. Time in two second intervals.

The afferent vasomotor fibres of the vagus. — It is well known that stimulation of the central cut end of the vagus often causes a rise of blood pressure. In our present experiments, after an efficient application of a solution of magnesium salt upon the vagus, we have frequently observed that a stimulation below the block would have no effect upon blood pressure, while stimulation above the block caused a distinct increase of it. Both afferent fibres of the vagus (respiratory and vasomotor) seem to have sometimes a different degree of irritability; and we could thus observe occasionally that a certain degree of stimulation below the block, which would have no effect at all upon the one kind of nerve fibres, would still produce some effect upon the other kind of fibres. More frequently, however, there would be still

some sort of a respiratory response to a stimulation below the block, while the blood pressure would remain unaffected, especially when the connection with the heart was severed. (See Fig. 2 *b*.)

The cardio-inhibitory nerve fibres. — The description given above of the effect of solutions of magnesium salts upon the respiratory fibres is thoroughly applicable also to the effects of these solutions upon the efferent fibres of the vagus causing inhibition of the heart. Application of a solution of magnesium sulphate or magnesium chloride to the intact or cut vagus nerve will produce sooner or later a more or less complete block in the peripheral direction of the nerve, so that in some cases a certain degree of stimulation applied on the vagus centrally to the anæsthetized area will produce no effect, while when applied peripherally to the block the same strength of stimulus would be sufficient to completely stop the heart beats. Such a block could be removed again by removing the solution and especially by washing the nerve with Ringer's solution. As in the case of the respiratory fibres, the solutions seemed to affect mainly the conductivity of the nerve fibres; when applying the electrodes directly to the block the effect would increase with the approach of the electrodes to its lower border.

The heart beats were studied in the majority of the experiments by recording the blood pressure in the usual manner. For an unmistakable reaction we relied chiefly upon a nearly complete stop of the heart beat expressed by a sudden considerable drop in the blood pressure. In a few experiments, especially when the behavior of the respiration was the chief consideration, the action of the heart was observed by a needle thrust into it.

Simultaneous blocking of cardiac and respiratory impulses. — In many of these experiments, in fact, in most of them, the behavior of the respiration and of the cardiac action was studied simultaneously. In these experiments, as a rule, one vagus nerve was cut and the solution applied to the other intact nerve. The graphic records of the respiration and the blood pressure were written one above the other. The results described above for each function separately were now observed simultaneously. When a complete block developed, stimulation below the block would bring on inhibition of the heart with no effect upon respiration, and stimulation above would have a characteristic effect upon the respiration, and perhaps also upon the heart beats. Application of the electrodes upon the block itself would show some moderate effect upon both functions, increasing in one or

the other direction according to the greater nearness of the electrodes to the one or other border of the block.

In these experiments a difference in the behavior between the respiratory and cardiac nerve fibres presented itself. In a good many instances we came across conditions in which a stimulation above the block had apparently no effect at all upon the heart beats, while a stimulation below produced some reaction upon the respiration. At a first glance this difference would seem to support the far-reaching assumption that the magnesium solutions have a selective action, that they affect the cardiac inhibitory fibres more readily and more profoundly than the respiratory fibres. We have touched upon this point before, and we shall now try to enter into a further discussion of it.

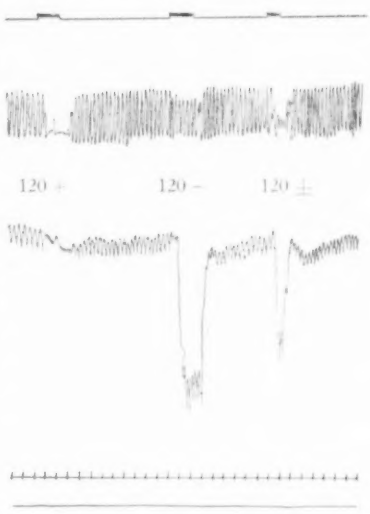

FIGURE 3. — Upper tracing respiration, inspiration down stroke; lower tracing blood pressure; upper line stimulation markings. After application of 3.7 per cent magnesium sulphate. 120 + = above, 120 − = below, 120 ± on the block. Time in three second intervals.

We wish to mention here, in the first place, that observations like those we have just mentioned were also made by W. E. Dixon [1] in studies of the effect of cocaine on nerve fibres. Among other experiments the vagus was painted by Dixon with a certain dilution of a cocaine solution, and the effect of stimulations upon respiration and cardiac inhibition were studied simultaneously. When the vagus was stimulated above the cocainized place the respiration was affected, but not the heart beats, while stimulation below affected heart as well as respiration, which, according to Dixon, shows that the employed solution of cocaine paralyzed the cardiac fibres, but not the respiratory nerve fibres of the vagus. Dixon looks upon this fact as a demonstration of the selective action of cocaine on nerve fibres, "picking out some fibres and paralyzing them before or to the exclusion of the others." As to the explanation of this selective activity of cocaine

[1] W. E. DIXON: Journal of physiology, 1905, xxxii, p. 87.

Dixon sees only three possible hypotheses: 1. Such a distribution of the fibres within the vagus as to expose one kind more than the other to the action of the drug. 2. That the different kinds of fibres are protected by different kinds of sheaths. 3. That one kind of fibres has a greater chemical affinity than the other for cocaine. Dixon decides in favor of the last hypothesis.

Our observations with magnesium salts were, as stated above, similar to those made by Dixon with cocaine. Some of the curves we have obtained were similar to those reproduced by Dixon from his experiments. But we never thought of assuming that our solutions affect the different nerve fibres in a different way. We believe that the difference has its origin in the different degrees of irritability of the two kinds of nerve endings. The irritability of the cardiac fibres is less than that of the respiratory nerve fibres. In the vast majority of our experiments we have found that a distinctly stronger stimulus is required to affect perceptibly the efferent cardiac fibres than the respiratory nerve fibres of the vagus; that is, many a stimulus which is sufficient to bring out a distinct respiratory effect is still incapable of producing a perceptible change in the cardiac beats. In making our interpretation we have also to bear in mind that with the increase in the strength of the stimulus the respiratory effect is not only increased quantitatively, but, as already stated above, is frequently also changed in character. Now we have found that, when by applying the solutions to the vagus a certain block was established for the cardiac fibres, there was invariably also some change in the effect upon the respiration, a change which corresponded to that produced by a stimulus of a lower strength. Our explanation then is simply this: a reduction in the conductivity in any nerve fibre, which practically means a reduction in the irritability, increases the threshold value or the minimum requirement of the stimulus to produce a certain effect, so that to obtain a similar effect as was obtained before the reduction, a much stronger stimulus has to be employed, or when the same stimulus is employed either there would be no effect at all, or the obtained effect would be of less value than the previous one; this is what had occurred indeed in our experiments: when the impulses to the heart were blocked the impulses to the respiration were either also completely blocked, or the effects upon the respiration were from below the block distinctly less than those obtained from above the block. In no case has it ever occurred that the effects upon respiration from above and from below were exactly the same when the impulses to the heart were distinctly blocked.

Obviously the same interpretation could be given to the similar phenomena observed by Dixon after the applications of cocaine upon the vagus as well as upon the other nerve fibres. However, a discussion of this general character is outside of the scope of this paper, and we shall not enter upon it.

A few of the statements made above on the effect of solutions of magnesium salts upon the respiratory and cardiac nerve fibres contained in the vagus are illustrated by a few graphic records. In Fig. 1, *A* was obtained a few minutes after a 25 per cent solution of magnesium sulphate was applied to the intact right vagus. The nerve was stimulated by an induction current with a coil distance of 100 millimetres. The minus (−) sign means that the electrodes were applied below, peripherally to the block, and the plus (+) sign means application above, centrally to the block. When stimulated below there was practically no effect, while from above an inspiratory standstill was produced. *B* was obtained a few minutes after the removal of the magnesium solution and the subsequent application of Ringer solution to the anæsthetized part of the nerve. A stimulation below with the same strength of current now produced an increase in the rate of respiration with slight shortening of the amplitude. In Fig. 2, *A* was obtained about ten minutes after the application of a 25 per cent solution of magnesium sulphate to the intact left vagus. From above (120+) the stimulation produced an inspiratory standstill and a rise of blood pressure, but with no effect at all upon the heart; from below there was a pronounced cardiac inhibition, but also a distinct effect upon the respiration. The degree of the effect upon the respiration,

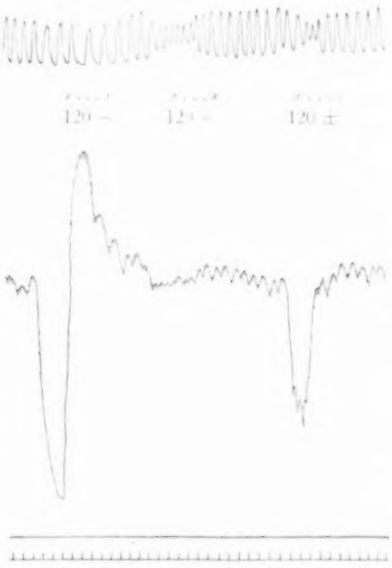

FIGURE 4. — Upper tracing respiration, inspiration up stroke; lower tracing blood pressure. 120 − = below, 120 + = above, 120 ± = on the block. Time in two second intervals.

however, was lower than that obtained from above and of another character; it was rather of an expiratory type. The effect upon the blood pressure was possibly covered by the cardiac effect. *B* was obtained later after a fresh application of the solution and after the vagus was ligated between the block and the heart. Now a stimulation below the block (but above the ligature) produced only a very slight effect upon the respiration with no effect at all upon the blood pressure, while from above the effect remained the same as before. The tracing of Fig. 3 was obtained after the intact nerve was thoroughly bathed with a 3.7 per cent solution of magnesium sulphate (equimolecular with 0.9 per cent sodium chloride). The sign (±) means that the electrodes were lying on the anæsthetized area. From above an inspiratory standstill with no effect on the heart was produced (except, perhaps, a systolic shortening?), neither was there in this case any effect upon the blood pressure. From below there was a strong inhibitory effect upon the heart with only very little effect upon the respiration (slight increase in rate and slight shortening). From the block itself both heart and respiration were distinctly influenced, but the effects were of lesser extent than from above or from below.

The tracing in Fig. 4 was obtained from the intact vagus about twenty minutes after the application of a 1.7 per cent solution of magnesium sulphate (hypotonic solution, equimolecular with 0.4 per cent sodium chloride). From above there was an increase in rate of respirations and considerable shortening of amplitude, with practically no effect upon the heart. From below there was distinct cardiac inhibition with a slowing of the respirations of an expiratory character. From the block itself there were again as in Fig. 3 moderate effects upon both functions. The tracing in Fig. 5 was obtained after application to the intact nerve of a hypotonic solution of 1.3 per cent of magnesium chloride (about equimolecular with 0.4 sodium chloride). Stimulations above affected the respiration by increase in rate and considerable shortening, with practically no cardiac inhibition. Stimulations below caused a marked cardiac inhibition while the effect upon the respiration was slight and consisted in a moderate slowing of an expiratory character.

Nerve fibres of œsophagus and cardia. — Not many experiments were made in studying the anæsthetizing effect of solutions of magnesium salts upon these fibres, but these few were instructive. A block could be produced with concentrated solutions (25 per cent magne-

sium sulphate) as well as with very dilute solutions (1.7 per cent magnesium sulphate). Fig. 6 *A* was obtained a few minutes after the application of a 25 per cent solution of magnesium sulphate to the left intact vagus (the upper tracing was obtained by means of a catheter, the fenestrated end of which was placed deep in the œsophagus while the other end was connected in the usual manner with a Marey's tambour. The elevations mean a contraction of the œsophagus). When stimulated above (160 +) there was a rise of blood pressure with no contraction of the œsophagus and with no inhibitory effect upon the heart, while when stimulated below (160 —) there was a contraction of the œsophagus and a distinct though moderate effect upon the heart.

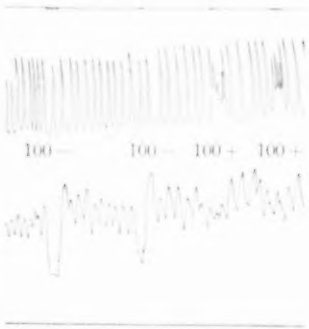

FIGURE 5. — One-third the original size. Upper tracing respirations, inspirations up stroke; lower tracing blood pressure. Upper line stimulation marking. After application of 1.3 per cent magnesium chloride. 100 — = below, 100 + = above block.

When applying dilute solutions the block could be effected only for stimulations with currents of moderate strength; strong stimuli would break through the block. Dilute solutions were here as in the other experiments more readily washed out again than concentrated ones.

The slow process of washing out concentrated solutions brought out here the interesting phenomenon that at a certain phase the nerve fibres of the œsophagus would have already regained their conductivity while the cardiac fibres would still be blocked. Our interpretation of this phenomenon is here again that it is not the result of an elective activity of the solution of magnesium salts but that it is due to the fact that the nerve fibres of the œsophagus possess a higher irritability than the cardiac inhibitory nerves. Fig. 6, *B* was obtained from the same vagus as Fig. 6 *A* but before the application of the solution. It shows that stimulation by a current of 250 coil distance, which produces a good effect upon the œsophagus, has no effect upon the heart; on the contrary it gives rise to a slight elevation of blood pressure.

An interesting observation on the effect of blocking the vagus was made upon the contractions of the œsophagus and cardia after deglu-

tition. Concentrated solutions of magnesium sulphate were applied to both intact vagi in the neck, and the animal was induced to swallow. After the block was established œsophagus and cardia did not participate in the act of deglutition. The contractions of the cardia were the first to disappear, then the contractions of the œsophagus followed. The entire œsophagus from the larynx down became soon perfectly relaxed. After "washing" both vagi with Ringer, contractions of the œsophagus returned first and those of the cardia later; the tonicity of the œsophagus also returned.

The depressor nerve. — Only two experiments were made and both with very dilute solutions. In one experiment 1.7 per cent magnesium sulphate was used and in the other a mixture of two parts of 1.3 per cent magnesium chloride with one part of 0.9 per cent sodium chloride was applied. In both experiments the blocking was distinct and the washing was promptly effective.

The cervical sympathetic nerve. — About half a dozen experiments were carried out. Only the effect upon the pupils was observed. Here again it can be summarized briefly that applications of strong concentrations as well as of hypotonic solutions of both magnesium salts produced invariably a more or less distinct block which was quite readily washed out again with Ringer solutions.

The crural nerve. — A few experiments were made upon this nerve with regard to the effect upon the sensation of pain. The results were not very satisfactory. We succeeded well enough by the application of the solutions to abolish all sensations of pain, but we failed mostly in the protection of the central portion from the narcotizing effect of the solutions, so that after their application we usually could produce no pain from any point of the exposed part of the nerve. Neither were we successful in most of the experiments in removing the block by washing with Ringer. However, we made altogether only a few experiments on this nerve, and did not study it with particular care. We should, however, mention the fact that the application of a strong solution to the fresh nerve never gave signs of the production of any kind of irritation.

The sciatic nerve. — The effects upon the motor and sensory nerve fibres within this nerve were studied simultaneously. Altogether not many experiments were made and not so many details and variations were elicited as in the extensive studies which we made upon the vagus. However the essential point was established, namely, that solutions of magnesium salts are capable of producing a block even

in such a thick nerve as the sciatic, and this with a solution of 3.7 per cent of magnesium sulphate or of 3.1 per cent magnesium chloride, which are about equimolecular with 0.9 per cent sodium chloride. It usually required a longer period for the solution to produce a perceptible block in this nerve than in the other nerves we have experimented upon. In one experiment the cotton saturated with the solution was left around the nerve for twenty-four hours and when tested later it was found that the block still persisted for the same

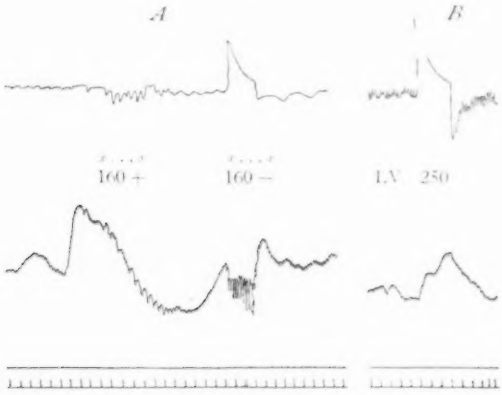

FIGURE 6. — Upper tracing œsophagus, lower tracing blood pressure. "*B*" obtained before application of magnesium. L. V. 250 = left vagus with 250 mm. coil distance. "*A*" obtained a few minutes after application to the vagus of 25 per cent magnesium sulphate. 160 + = stimulated above, 160 − = stimulated below block, with 160 mm. distance.

strengths of current as the previous day. In another experiment in which, after a block was established, the cotton was removed and the wound sewed up, it was found after twenty-four hours that the conductivity of the nerve was completely restored.

In the experiments on the sciatic nerve it was found that the motor nerve fibres have shown somewhat greater resistance to the narcotizing effect of the magnesium salts than the sensory nerve fibres; that is, with some currents it was found that they produced from below the block contractions and no pain, while from above there was pain and some contraction. Here again our interpretation of this phenomenon is that the motor nerve fibres possess a greater irritability than the sensory fibres, and correspondingly we have found that a certain moderate stimulus which when applied to the

sciatic would not cause any pain yet would produce well marked contractions of the muscles of foot and leg.

The superior cervical ganglion. — The fact that the block produced in a nerve by prolonged application of a concentrated solution of magnesium salts proved resistant to removal by washing, induced us to try the following experiment: In a rabbit the superior cervical ganglion was isolated and well exposed and surrounded by absorbent cotton. For many hours the cotton was frequently saturated with a 25 per cent solution of magnesium sulphate and then removed and the wound sewed up. It is now known that twenty-four hours after the removal of the superior cervical ganglion a subcutaneous injection of adrenalin will cause a long-lasting dilatation of the pupil of the corresponding side.[1] When injected sooner or when injected into a normal animal the pupil remains unaffected by adrenalin. We have presumed then that the prolonged application of the solution might produce a complete narcotization of the ganglion which would possibly last twenty-four hours and longer. In one experiment the expectation was realized; twenty-four hours after the treatment of the ganglion with the magnesium solution an injection of adrenalin produced a dilatation of the pupil; a repetition of the procedure twenty-four hours later had no effect. In this case the saturation of the ganglion with the magnesium acted like a removal and the effect lasted more than twenty-four hours; after forty-eight hours, however, conductivity and irritability were re-established again. In another experiment, however, this procedure brought no success; at no time did the pupil dilate after a subcutaneous (or intramuscular) injection of adrenalin. In this case either the applications produced no complete anæsthesia or the ganglion recovered its irritability and conductivity in less than twenty-four hours.

Summary.

Solutions of magnesium salts, even in strong concentrations, when directly applied to the nerve trunks of animals, never seemed to produce irritation.

By applications of solutions of magnesium salts to nerve trunks the conductivity can be interrupted, a more or less complete block for afferent and efferent, for normal or artificial, impulses, can be established. This can be accomplished by hypertonic, isotonic, and

[1] S. J. Meltzer and Clara Meltzer Auer: This journal, 1904, xi, p. 28.

hypotonic solutions. The more concentrated the solution the sooner the effect is established. In general it takes more time for the magnesium solutions to cause a nerve block than for other known local or general anæsthetics. The block produced in the nerves by the magnesium solutions disappears some time after removal of the solutions; the recovery of the nerve is greatly assisted by washing with Ringer's solution.

The solutions of magnesium salts affect the cardiac fibres more readily than the efferent fibres for the œsophagus and the afferent respiratory and vasoconstrictor fibres within the vagus; also the sensory fibres within the sciatic nerve are more readily affected than the motor nerve fibres. It is believed that the difference is not due to a selective action of the magnesium solutions upon the different nerve fibres but to a difference in the irritability of the nerve endings of these nerve fibres.

THE ELIMINATION OF CREATININ.

By OLIVER E. CLOSSON.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

THE significance of creatinin as a product of metabolism has always been rather obscure. The experimental evidence that the creatinin of the urine may be derived in part at least from creatin or creatinin ingested, particularly in the form of flesh foods, is reasonably conclusive. The problematic features of the physiological history of urinary creatinin involve especially its relation to the metabolism of muscle tissue and its possible origin from proteids and other nitrogenous compounds. Regarding the influence of pathological conditions upon the production or elimination of creatinin the published data have been even more conflicting and untrustworthy. For this the lack of a thoroughly satisfactory and reliable method of analysis until recently has been largely responsible.

In 1900 Long [1] published analyses of the urines of seven vegetarians, students of the American Medical Missionary College, who for more than a year had subsisted upon a diet of bread, fruits, vegetables, and prepared cereal and nut foods exclusively, *i.e.*, upon practically a creatin-free diet. Eggs were not eaten, and only as much milk as was taken with the "cereal coffee" used. The creatinin output, measured by the Salkowski-Neubauer method, averaged as a mean of twenty-four analyses 0.8 gm. per day with an average daily urine volume of 874 c.c. and urea output of 28 gm. The individual values fluctuated very widely, *e.g.*, from 0.087 to 1.58 gm. of creatinin per litre of urine, without equally large variations in the urea output. No data were furnished regarding the body-weights of the subjects. Long suggests that the low figures are probably due to low food consumption and metabolism. The men furnishing the urine performed little muscular work and took little exercise. The mean results for daily excretion obtained by the same author [2] from twenty-four urines of individuals living on a mixed diet of meat and vegeta-

[1] LONG: Journal of the American chemical society, 1900, xxii, p. 592.

[2] LONG: *Ibid.*, p. 372.

bles indicate a volume of 1167 c.c., urea 30 gm., creatinin 1.6 gm., likewise with considerable range between the individual figures.

Caspari and Glæssner,[1] on the other hand, have reported careful investigations of the metabolism of two vegetarians accustomed for several years to a creatin-free diet. The urine obtained on a dietary including sugar, coffee, dates, nuts, oil, potatoes, carrots and crackers, contained *no creatinin whatever*, according to analyses by the Salkowski-Neubauer method. Creatin was present and, estimated by the very unsatisfactory method of Voit and Meissner, indicated amounts averaging:

In the 69 kilo man	0.111 gm. per day
In the 58 kilo woman	0.074 gm. per day

The authors suggest that the replacement of creatinin by creatin in the urine may be a characteristic of the vegetarian régime.

To these illustrations of the divergence of opinion regarding the output of creatinin after meat-free diets many other cases might be added. Thus Macleod[2] determined an *endogenous* excretion of 1.06 gm. per day and failed to find any diminution in a case of muscle atrophy. The few reports regarding the elimination of creatinin in starvation are likewise somewhat discordant, though they tend to show a gradual decrease in the output.[3]

Since plants presumably contain no creatin or creatinin, while both urine and tissues of herbivora contain these compounds, it seems certain that we may properly speak of *endogenous* and *exogenous* creatinin elimination in the same sense as these terms are now used in reference to purin metabolism. This nomenclature furnishes no explanation of the actual origin of the endogenous components. The preponderant mass of muscle tissue rich in creatin in the case of vertebrates naturally claims first attention. Koch[4] has recently advanced the hypothesis that creatinin is an index of methyl metabolism in the body and believes that under ordinary conditions of diet the methyl groups of the lecithin and kephalin ingested can all be accounted for by the methyl groups of the creatinin excreted. He admits, however, that further experiments will have to determine if physiological activity is capable of influencing this metabolism to a

[1] Caspari and Glaessner: Zeitschrift für diätetische und physikalische Therapie, 1903–04, vii, Heft 9.

[2] Macleod: Journal of physiology, 1900, xxvi, p. vii.

[3] *Cf.* Weber: Ergebnisse der Physiologie, 1902, i, I, p. 710; Van Hoogenhuyze and Verploegh: Zeitschrift für physiologische Chemie, 1905, xlvi, p. 415.

[4] Koch: This journal, 1905, xv, p. 15.

greater extent than the presence of an excess of methyl groups in the form of lecithin and kephalin.

The most noteworthy contribution to this subject are the observations of Folin[1] showing the remarkable constancy with which creatinin is excreted in man under widely varying conditions of diet. This author regards urinary creatinin as by far the most reliable index to the amount of a certain kind of proteid metabolism occurring daily in any given individnal. In his own words "the absolute quantity of creatinin eliminated is a constant quantity different for different individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated." Folin has noted that the chief factor in determining the amount of creatinin elimination appears to be the weight of the person, although the proportion between the body-weight and amount of creatinin in the urine is not very constant. Corpulent persons yield less per unit of body-weight than lean ones, average figures being 20 mgm. of creatinin per kilo for the former, while the lean persons yield about 25 mgm. This suggests that the bulk of the muscle tissue plays a significant rôle, as Burian[2] believes it does in endogenous purin metabolism.

Van Hoogenhuyze and Verploegh[3] have supplemented Folin's investigations with additional data. They agree in finding a parallelism between the output of urea and the intake of proteid, without any corresponding variations in the elimination of creatinin. Muscular work was without influence on the absolute amount of urinary creatinin, except in the case of a fasting subject in whom exercise on the tenth day of inanition was followed by an undoubted rise in the creatinin output on the succeeding days. Here it is to be noted, however, that the metabolism of the nitrogenous compounds is undoubtedly singularly modified in starvation, the absolute quantities of otherwise constant endogenous katabolites like uric acid and creatinin being greatly diminished. The essential features of Folin's contention regarding the constancy of endogenous creatinin elimination, despite varying nitrogenous intake of creatin-free food, has also received verification by Klercker.[4] This author maintains, however, that our present ideas regarding the exogenous factors require revision. His

[1] FOLIN: This journal, 1905, xiii, pp. 84, 118.

[2] BURIAN: Zeitschrift für physiologische Chemie, 1905, xliii, p. 532.

[3] VAN HOOGENHUYZE and VERPLOEGH: Zeitschrift für physiologische Chemie, 1905, xlvi, p. 415; also PEKELHARING: Koninklije Akademie van Wetenscappen te Amsterdam, 1905, September 30.

[4] KLERCKER: Hofmeister's Beiträge zur chemischen Physiologie, 1906, viii, p. 59.

preliminary studies suggest that creatin and creatinin are somewhat independent of each other in their metabolic transformations, each being eliminated again in large part as such, and not exclusively in the form of creatinin, as has hitherto been assumed. There is, undoubtedly, need of considerable further investigation along these lines. Noel Paton [1] has reported a few observations on a dog kept under widely varying conditions of diet during eleven days and concluded that in this animal there is a relationship between the production of creatinin and the intake of nitrogen. Under more constant conditions Koch [2] found little variation in the daily average over long periods, a result confirmed by many observations lately made on dogs in this laboratory.

The first opportunity for the present experiments was afforded by an extensive series of nutrition investigations on man, conducted in this laboratory two years ago.[3] Some of the subjects were living upon a meat-free (creatin-free) diet and several were upon a somewhat lower "plane of nitrogenous equilibrium" than is customarily adopted by the average individual. Professor Mendel therefore suggested the desirability of studying the output of creatinin in the urine under a so-called vegetarian régime and the influence of marked variations in proteid intake and katabolism on this same factor. We have also had opportunity to investigate creatinin elimination in a number of other conditions.

In our earliest experiments the Salkowski-Neubauer method, by which creatinin is estimated as the zinc chloride compound, alone was available. The unsatisfactory character of this reaction, the difficulty in obtaining good duplicate analyses and the uncertainty regarding the best conditions for reliable results are familiar to all analysts. Very lately Van Hoogenhuyze and Verploegh [4] have published an effective criticism of the method. These writers have subjected the simple colorimetric method of Folin,[5] used in nearly all of our trials, to an experimental investigation and commend it as most satisfactory. We have employed the method precisely according to the directions of Folin, using the colorimeter of Duboscq as

[1] NOËL PATON: Journal of physiology, 1905, xxxiii, p. 1. The conclusions are criticised by VAN HOOGENHUYZE and VERPLOEGH.

[2] KOCH: *Loc. cit.*

[3] *Cf.* CHITTENDEN: Physiological economy in nutrition, 1904.

[4] VAN HOOGENHUYZE and VERPLOEGH: Zeitschrift für physiologische Chemie, 1905, xlvi, p. 415.

[5] FOLIN: *Ibid.*, 1904, xli, p. 223. The method has also been recommended by KLERCKER: Hofmeister's Beiträge zur chemischen Physiologie, 1906, viii, p. 59.

recommended. In several cases comparable figures obtained by the Folin and the Salkowski-Neubauer methods were concordant: usually, however, the latter gave lower values.

Experimental.

I. **Vegetarian dietary.**—Data were obtained at various times from two individuals who had been accustomed to a so-called vegetarian diet for a considerable period. The less reliable and somewhat variable figures obtained by the Salkowski-Neubauer method will not be reported here, only those afforded by Folin's method being recorded below.

1. G.M.B. Age thirty-eight; somewhat frail in physique. This subject was under observation for many months, the protocols of dietary, etc., being presented in detail in Chittenden's *Physiological Economy in Nutrition*, p. 109 flg. He had tasted practically no meat, eggs, or fish during the fifteen months previous to the days on which the following analyses were made.

COMPOSITION OF THE URINE. (Body-weight, 61.5 Kilos.)

Date.	Volume.	Specific gravity.	Nitrogen.	Creatinin.
1904	c.c.		grams	grams.
April 18 . .	610	1.025	7.69	1.05
" 19 . .	870	1.020	9.87	0.89
" 20 . .	985	1.019	8.87	0.99
" 21 . .	795	1.024	8.25	1.11
" 22 . .	1090	1.019	9.02	1.23
" 23 . .	970	1.022	8.44	1.12
" 24 . .	810	1.021	7.30	0.94
" 25 . .	850	1.018	6.94	1.29
" 26 . .	620	1.024	7.11	1.26
" 28 . .	450	1.027	8.58	1.33
" 29 . .	650	1.025	9.52	1.30
" 30 . .	1260	1.018	10.20	1.36
May 1 . .	1060	1.017	8.71	1.15
" 2 . .	610	1.025	8.16	1.23
" 3 . .	650	1.022	8.70	1.17
" 4 . .	870	1.019	8.51	1.26

2. L.B.M. This subject, age thirty-two, was not of corpulent build at the time when the experiments, recorded in detail in Chittenden's book, p. 52 flg., were in progress. He had abstained from meat, fish, and eggs for several months. Two periods of observation are here reported, in the second of which the income and outgo of nitrogen were accurately determined. The diet at this time included cereals and cereal foods, legumes, fruits, vegetables, sugars, and milk products. During the first period the uric acid output averaged 0.373 gm. per day; during the second, 0.325 gm., indicating in itself that the dietary was also practically purin-free. Professor Mendel had, at this time, been living on this low proteid intake for some months.

COMPOSITION OF THE URINE. (BODY-WEIGHT, 70 KILOS.)

Date.	Volume.	Specific gravity.	Nitrogen.	Creatinin.
	First Period.			
1904	c.c.		grams	grams
April 25	935	1.021	5.78	1.22
" 26	1000	1.021	6.18	1.31
" 27	1295	1.015	6.06	1.26
" 28	1425	1.013	5.56	1.30
" 29	990	1.022	6.24	1.30
" 30	1100	1.021	7.32	1.31
May 1	1380	1.014	5.96	1.22
	Second (Balance) Period.			
May 18	660	1.021	6.06	1.20
" 19	905	1.018	7.17	1.20
" 20	685	1.022	6.33	1.07
" 21	1142	1.018	6.78	1.09
" 22	1055	1.019	5.70	1.02
" 23	1053	1.018	5.75	1.09
" 24	895	1.020	6.39	0.98

Average intake of nitrogen per day	8.2 gm.
" total output of nitrogen per day	7.9
" daily nitrogen balance	+0.3 gm.

3. E.H.R. Professor Mendel has permitted me to report in this connection the results of a study of creatinin elimination made in his laboratory by Dr. E. H. Risley. The subject, a young physician of slender stature, had been accustomed for several years to an exclusive vegetarian régime, no food of animal origin whatever being consumed by him. During the experimental period the diet included cereal products, maltose, butter, nuts, and nut products, all of which were carefully analyzed. On May 9, 10, and 11, varying quantities of nutrose (sodium caseinogenate), containing 13.25 per cent of nitrogen, were added to the usual foods. It will be noted that a very wide range of nitrogen intake is here reported, and that during part of the days the proteid intake was insufficient to maintain nitrogen equilibrium.

COMPOSITION OF THE URINE AND FOOD. (BODY-WEIGHT, 57 KILOS.)

Date.	Nitrogen intake.	Urine volume.	Specific gravity.	Nitrogen in urine.	Creatinin.	Uric acid.
1905	grams	c.c.		grams	grams	grams
May 3 . .	5.44	400	1.032	5.46	1.09	0.39
" 4 . .	8.63	565	1.025	7.86	1.05	0.32
" 5 . .	8.55	545	1.024	7.32	1.12	0.38
" 6 . .	8.51	480	1.026	6.93	1.14	0.43
" 7 . .	8.37	400	1.030	6.96	1.08	0.35
" 8 . .	8.40	370	1.034	6.60	1.09	0.27
" 9 . .	11.83	470	1.031	9.75	1.10	0.43
" 10 . .	14.97	470	1.033	11.40	1.11	0.39
" 11 . .	13.76	500	1.033	12.03	1.11	0.40
" 12 .	8.80	610	1.023	9.00	1.09	0.37
" 13 . .	8.51	560	1.024	7.41	1.19	0.34
" 14 . .	8.54	540	1.024	7.20	1.11	0.34
" 15 . .	3.48	360	1.030	4.80	1.08	0.30
" 16 . .	2.50	380	1.028	4.74	1.09	0.26
Average . . .	8.58	475	1.028	7.67	1.10	0.35

The data obtained by Dr. Risley represent the most constant output of creatinin hitherto observed in this laboratory on any single individual. These results were obtained despite considerable variations in the total nitrogenous katabolism and correspond with the findings of Van Hoogenhuyze and Verploegh,[1] who likewise failed to obtain noteworthy fluctuations in the creatinin output when the total nitrogen elimination was considerably augmented by the addition of fifty grams of casein or gelatin to the customary diet. The results also differ from the variable elimination reported for the dog by Noël Paton.[2]

The observations on these three subjects leave no doubt that even after the long continued adoption of creatin-free dietaries the (endogenous) elimination of creatinin in the urine may still reach a considerable figure. The daily quantities excreted by the first two subjects vary little more than do the figures reported by Folin or Van Hoogenhuyze and Verploegh for normal persons. The individual constants are not widely different, thus:

	Body-weight, kilos.	Creatinin, mgm. per kilo.
L.B.M.	70.0	17.0
G.M.B.	61.5	18.8
E.H.R.	57.2	19.0

The last corresponds with Folin's Dr. A., weighing 56 kilos and eliminating 1.15 gm. per day, or 20.5 mgm. per kilo.

II. Restricted proteid diet. — In addition to the observations on the vegetarians of group I, all of whom were living on a rather low intake of proteid, the following figures were obtained from an individual who had, for over a year, been accustomed to a nitrogen intake of about 0.1 gm. per kilo and who was evidently in nitrogenous equilibrium at this level of metabolism.

The subject R.H.C., age forty-seven, was living on a mixed diet; but the amount of meat and creatin-yielding foods was obviously small. (For some of his dietaries, see Chittenden's book, p. 34 flg.)

[1] Van Hoogenhuyze and Verploegh: *Loc. cit.*, pp. 460, 463.
[2] Noël Paton: *Loc. cit.*

COMPOSITION OF THE URINE.

First Period. (Body-weight, 57 Kilos.)					
Date.	Volume.	Specific gravity.	Nitrogen.	Creatinin.	Uric acid.
1904	c.c.		grams	grams	grams
April 26	450	1.028	4.97	0.94	0.37
" 27	600	1.025	6.62	0.90	0.55
" 28	385	1.029	5.66	1.11	0.51
" 29	415	1.029	5.28	0.90	0.49
" 30	462	1.029	5.59	0.94	0.41
May 1	486	1.027	5.54	0.86	0.41
" 2	405	1.028	4.11	0.86	0.32
" 3	505	1.027	5.48	0.94	....
" 4	456	1.026	5.27	0.72	0.50
" 5	380	1.026	4.88	0.76	0.63

Second Period. (Body-weight, 58 Kilos.)				
Date.	Volume.	Specific gravity.	Nitrogen.	Creatinin.
1905	c.c.		grams	grams
Jan. 19 . .	520	1.026	5.80	0.88
" 20 . .	410	1.028	5.14	0.81
" 21 . .	400	1.029	5.14	0.82
" 22 . .	600	1.025	6.55	0.90
" 23 . .	535	1.027	5.68	0.95
" 24 . .	460	1.027	5.30	0.83
" 25 . .	415	1.027	5.18	0.74
" 26 . .	495	1.026	5.64	0.80

Third Period. (Body-weight, 59 Kilos.)				
1906				
April 3 . .	355	1.028	4.76	0.88
" 4 . .	310	1.027	5.00	0.80
" 5 . .	395	1.026	5.28	0.94

A comparison of the creatinin output of G.M.B., L.B.M., and R.H.C. shows a somewhat lower elimination per kilo of body-weight than has been reported by other investigators. It is to be remembered that these individuals had all been living for many months on quantities of proteid presumably approaching closely the minimum requirement for each.

The average daily output of nitrogen for the preceding six months or more is tabulated below with other data for comparison.

	Body-weight.	Average daily nitrogen in urine.	Average daily creatinin output per kilo of weight.	Average daily output of uric acid.
	kilos	grams	milligrams	grams
R.H.C. . .	57.5	5.4	15 to 16	0.390
G.M.B. . .	61.5	8.6	18 to 19	0.365
L.B.M. . .	69.5	6.5	16 to 18	0.420

To these data may be added a comparable series of observations on F.P.U. (Chittenden's book, page 76 fig.), age twenty-six years, who ingested meat in somewhat larger quantities than any of the preceding subjects. The marked variations in creatinin output in this case are doubtless due to changes in the quantities of meat eaten, particularly on April 25, 27, and 28. The remaining days furnish an average which doubtless approaches the endogenous figure for this person.

COMPOSITION OF THE URINE. (BODY-WEIGHT, 65 KILOS.)

Date.	Volume.	Specific gravity.	Nitrogen.	Creatinin.
1904	c.c.		grams	grams
April 25 . .	600	1.021	8.23	1.45
" 26 . .	610	1.014	5.82	0.96
" 27 . .	600	1.023	7.27	1.37
" 28 . .	970	1.015	7.05	1.42
" 29 . .	795	1.017	5.91	1.18
" 30 . .	700	1.021	6.80	1.08
May 1 . .	795	1.016	5.72	1.01

Finally, we may add a few analyses of the urine of O.E.C., twenty-four years of age, living four days on a creatin-free diet for the purpose of this experiment.

COMPOSITION OF THE URINE. (BODY-WEIGHT, 62.5 KILOS.)

Date.	Volume.	Specific gravity.	Nitrogen.	Preformed creatinin.	Total[1] creatinin.	Uric acid.
1904	c.c.		grams	grams	grams	grams
May 2 . .	820	1.038	9.2	1.53	1.54	....
" 3 . .	770	1.029	10.7	....	1.37	0.51
" 4 . .	754	1.029	10.5	1.28	1.32	0.56
" 5[2] . .	652	1.030	8.7	1.12	1.34	0.46

[1] By total creatinin is meant the figure obtained after heating the urine with acid to convert any creatin present into creatinin. It therefore represents the sum of the preformed creatinin and the creatin in terms of creatinin.

[2] These figures represent the output of twenty-three hours only.

III. The rate of elimination of endogenous creatinin. — In the hope of throwing some light on the relation of creatinin production to general nitrogenous metabolism, a number of experiments were made in which the urine was collected at brief intervals of two to four hours, and the comparative rate of creatinin and total nitrogen elimination thus ascertained. We were at first inclined to interpret the analytical data as indicating a close parallelism between these two factors. A more careful inspection of the figures obtained over several days on a creatin-free diet fails, however, to establish any such direct relation; and it appears more probable that the elimination of creatinin, like that of uric acid, is independent of the excretion of urea comprising the bulk of the nitrogen eliminated normally.[1]

The results obtained on O.E.C. are represented graphically in the chart on the opposite page.

IV. The elimination of creatinin in some unusual conditions. — 1. *Children.* — Physiological literature contains scarcely any references to the occurrence of creatinin in the urine during the period

[1] For the literature on the rate of uric acid elimination at brief intervals, see MENDEL: Journal of the American medical association, 1906, March 31. Similar data regarding creatinin elimination are also published by VAN HOOGENHUYZE and VERPLOEGH: *Loc. cit.*

of growth. Rietschel[1] failed to detect it in suckling infants by the Salkowski-Neubauer method, or by Weyl's reaction, except during febrile conditions, or when 5 to 10 gm. of meat extract were added to the diet. The author points out that the infants examined by him were all in a more or less weak condition (hospital patients), and suggests that inasmuch as the infant is not normally in nitrog-

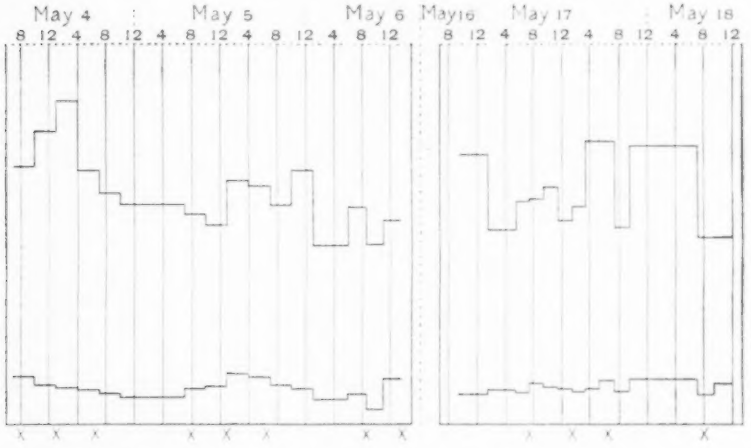

FIGURE 1. — Hourly elimination of total creatinin and total nitrogen estimated at brief intervals. Subject, O.E.C. Body-weight, 62 kilos. The divisions of the ordinate scale between the two charts represent quantities of 15 mgm. The abscissæ represent the duration of the experiments in hourly units. The meal times are indicated by an × below the base line. The total nitrogen output is represented in the upper line and the total creatinin output in the lower one. The parallel lines are drawn at intervals of four hours.

enous equilibrium, the conditions may resemble those noted in the nitrogen hunger during starvation, where the values are always lowered. Rietschel adds: "Bei dieser Auffassung konnen wir beim Stoffwechsel im Kreatinin ebenso das Produkt eines spezifischen Muskelstoffwechsels sehen, der, wie der der Harnsaure, und der Purinbasen, dem eigentlichen Eiweissstoffwechsel als koördiniert anzureihen ist, aber von ihm unabhängig im Gesammtstoffwechsel einhergeht." Van Hoogenhuyze and Verploegh,[2] using Folin's method, have succeeded in finding creatinin regularly in the urine

[1] RIETSCHEL: Jahrbuch für Kinderheilkunde, 1905, lxi. p. 615.
[2] VAN HOOGENHUYZE and VERPLOEGH: *Loc. cit.*, p. 440.

of sucklings. Incidentally they noted that the quantities were considerably smaller in the case of a weak child nourished with cow's milk than in healthy breast-fed infants.

Our own estimations are confined to two boys:

A.C. Body-weight, 22 kilos; age, six years and nine months. He was convalescent from endocarditis at this time, living on a prescribed meat-free diet, and taking little exercise.

COMPOSITION OF URINE

Day.	Volume.	Nitrogen.	Creatinin.[1]	Uric acid.
	c.c.	grams	grams	grams
1	600	5.9	0.42	0.25
2	860	7.4	0.32	0.21

[1] The creatinin was determined by the Salkowski-Neubauer method in this experiment.

P. Body-weight, 27.5 kilos; age, fourteen years. This boy had been a patient under the care of Professor Osborne for some time, and represented a case of diabetes insipidus. On a diet of vegetables, milk, apples, pastry, and coffee, he eliminated, on a typical experimental day, 2510 c.c. of urine, having a specific gravity of 1.004, with 6.1 gm. of nitrogen, 1.3 gm. of P_2O_5, and 0.44 gm. of total creatinin.

2. *Observations on suckling animals.* — We have made a few experiments on the urine of very young puppies and kittens which indicate the early appearance of creatinin in the urine of these species. Thus in the secretion of a puppy one week old Jaffé's test was very distinct; and in the fresh urine of a kitten one and one-half weeks old, 0.02 per cent of preformed creatinin and 0.04 per cent total creatinin (creatin and creatinin) were found. The following data on sucklings' urine also show the preformed creatinin to be considerably less than the total creatinin and much more variable.

Three puppies from the same litter were confined in one cage and the urine collected at intervals. The diet during this period consisted of milk. Similarly two kittens on a milk diet were subjected to observation. The figures below are averages calculated per animal per day.

Age in weeks.	Body-weight.	Nitrogen.	Creatinin.	Total creatinin.
	PUPPIES.			
	grams	grams	grams	grams
3	750	....	0.001	0.018
6	1300	0.55	0.018	0.021
6½	1380[1]	0.95	0.011	0.037
8½	1700[1]	0.76	0.019	0.048
	KITTENS.			
4	750	0.48	trace	0.013
4½	....	0.54	0.006	0.015
5	....	0.60	0.011	0.021
5½	....	0.54	0.010	0.023

[1] These data represent analyses from a single animal.

3. *Acromegaly.* — A series of observations made on a case of a pronounced type are of interest here because the diet was carefully regulated and various other features were studied by Professor Mendel.

The patient was a young man of about thirty-five years, weighing over 90 kilos. The records cover three periods, during two of which special attention was paid to the endogenous output of uric acid. The diet at this latter time was both purin- and creatin-free.

SUMMARY OF OBSERVATIONS.

Date.	Volume.	Specific gravity.	Nitrogen.	Uric acid.	Total creatinin.	P_2O_5.	Remarks.
	I.						
1904	c.c.		grams	grams	grams	grams	
Apr. 26	1300	1.020	16.4	1.08	1.84	3.05	
" 28	1730	1.014	13.9	0.97	2.30	1.99	Body-weight, 92.5 kilos. Mixed diet including meat.
" 29	910	1.025	12.9	1.10	2.57	1.84	

SUMMARY OF OBSERVATIONS. (*Continued.*)

Date.	Volume.	Specific gravity.	Nitrogen.	Uric acid.	Total creatinin.	P_2O_5.	Remarks.
	II.						
1904	c.c.		grams	grams	grams	grams	
May 1	970	1.018	8.8	0.63	1.71	1.80	Creatin-free and practically purin-free diet.
" 2	790	1.021	9.2	0.79	1.88	1.45	
" 3	941	1.020	8.6	0.75	2.00	1.80	
	III.						
June 12	908	1.026	10.3	0.90	1.80	2.24	Body-weight, 90 kilos. Diet strictly purin- and creatin-free.
" 13	1280	1.021	10.7	0.74	1.85	3.27	
" 14	903	1.027	10.7	0.81	2.00	2.66	

Here, too, a striking constancy in the output of total creatinin on a creatin-free diet is observed, the large figures corresponding, in accord with Folin's hypothesis, with a large mass of body-tissue in the 90 kilo subject. The relatively high output of endogenous uric acid on a purin-free diet was a noteworthy feature of this case.[1] The large output of P_2O_5 in the third period can be traced to the milk taken, and is thus of uncertain significance.

V. Summary and conclusions. — Our observations clearly indicate that creatinin is an unfailing constituent of the urine from an early period in man, the dog, and the cat; and, independent of the ingestion of creatin and creatinin, it forms a characteristic endogenous, katabolic end-product. The experimental data contributed in this paper are in accord with observations of Folin and others in showing a fairly marked uniformity in the quantity of endogenous creatinin eliminated by any individual under variable conditions of diet. Furthermore, they are not essentially at variance with Folin's opinion that the excreted creatinin is quantitatively dependent upon the mass of the active body tissues, and therefore to a certain degree proportional to the body-weight. Arranged in order of this factor, the fol

[1] *Cf.* Rockwood: This journal, 1904, xii, p. 38.

lowing summary of average figures can be drawn from our protocols regarding endogenous creatinin:

Subject.	Body-weight.	Creatinin.	Creatinin per kilo.
	kilos	grams	mgm.
A.C.	22.0	0.37	17
P.	27.5	0.44	16
E.H.R.	57.2	1.10	19
R.H.C.	57.5	0.87	15
G.M.B.	61.5	1.17	19
O.E.C.	62.5	1.46[1]	23[2]
F.P.U.	65.0	1.21	19
L.B.M.	70.0	1.18	17
Acromegaly	90.0	1.87	21[2]

[1] This figure is the average of data from three days besides those recorded above.
[2] Represents the total creatinin per kilo.

The figures for the mean values of the daily excretion of creatinin are, perhaps, somewhat lower than the data of Folin lead one to expect, most noticeably, perhaps, in those adults, R.H.C. and L.B.M., who were living exceptionally close to the minimal proteid requirement for man. In these cases, however, a much lower daily output than actually noted might have been expected if, as some have assumed, creatinin elimination is more directly proportionate to the total nitrogenous metabolism.

Finally, a few records have been presented to indicate the rate of elimination of endogenous creatinin in contrast with that of the total nitrogen of the urine in brief periods; and a number of data afforded by exceptional cases have been discussed.

A STUDY OF THE METABOLISM AND PHYSIOLOGICAL EFFECTS OF CERTAIN PHOSPHORUS COMPOUNDS WITH MILCH COWS.

By W. H. JORDAN, E. B. HART, and A. J. PATTEN.

[*From the Chemical Laboratory of the New York Agricultural Experiment Station.*]

Introduction.

THE metabolism and functions of the ash ingredients of cattle foods have received relatively little attention from scientific investigators up to the present time. This is true even of so important an element as phosphorus. So far, investigators have given their attention chiefly to studies of the metabolism and functions of the compounds included in the three classes, proteids, carbohydrates, and fats. There is every reason for believing, however, that some of the elements which appear in plant ash sustain just as important practical relations to animal production of various kinds as do nitrogen and carbon in their various combinations.

The purpose of the investigations herein reported has been to study some of the nutritive relations and functions of the phosphorus compounds of cattle foods. The results reached in the preliminary stages of this work have already been reported in bulletins Nos. 238 and 250, in which certain conclusions as to the status of phosphorus in feeding stuffs are set forth.

The problems studied. — Our problem, as first conceived, was the narrow one of the food compounds used by the animal for the formation of the phosphorus-bearing proteids such as are found in flesh, milk, and eggs. It was thought, if nucleo-proteid synthesis does not occur in the animal body, that in the case of cows and hens where phosphorus-bearing proteids are formed in great abundance in the milk and eggs, the kind and quantity of phosphorus compounds in the ration might have an important influence upon production. As it was believed that cattle and poultry foods probably contained both inorganic and organic phosphorus bodies in greatly varying relative

and absolute quantities, it was supposed that rations might be selected which would show markedly different effects upon the secretion of phosphorus-bearing proteids, and therefore upon production with cows and hens, milk and eggs being rich in these bodies.

Our views of the conditions attending our proposed investigation were much modified by an examination into the nature of the phosphorus compounds of the various grains and their by-products. In Bulletin No. 238 of this Station[1] it was first shown, and later confirmed by Schulze and Castoro,[2] that in normally grown non-etiolated plants the phosphorus exists in the seeds wholly in organic combinations, while in the straw a small proportion occurs in inorganic forms. It was also shown in a later bulletin[3] that wheat bran, the outer layer of the wheat kernel and rich in phosphorus, contains practically all of this element in an organic combination of an entirely different nature from either the nucleins or lecithins.

This bran body is an organic acid radical coupled with calcium, magnesium, and potassium, and is probably identical with the compound Posternak[4] isolated from other plant tissues, and to which he gave the name of phytin.[5] Phytin is widely distributed in nature, and has been found in the seeds of red fir, pumpkin, peas, beans, white and yellow lupines, potatoes, and wheat.

It can be completely or nearly removed from wheat bran by mere washing with water, but more easily by allowing the bran to undergo a slight acid fermentation followed by leaching with water. It is this fact that allowed us to prepare a ration very low in its phosphorus content.

General Plan of the Investigation.

Our studies for the past two years have been conducted with milch cows, and in connection with observations that are to follow, have been arranged in accordance with the following general plan:

1. Feeding to the same animal, during short or long periods of time, rations differing greatly in the amount of phosphorus which

[1] New York Agricultural Experiment Station, Bulletin 238, 1903, and American chemical journal, 1903, xxx, p. 470.

[2] Schulze and Castoro: Zeitschrift für physiologische Chemie, 1904, xli, p. 477.

[3] New York Agricultural Experiment Station, Bulletin 250, 1904, and American chemical journal, 1904, xxxi, p. 564.

[4] Posternak: Revue générale de botanique, 1900, xii, p. 5.

[5] Schweizerische Wochenschrift für Pharmacie, 1904, xlii, p.

they supply, this variation in phosphorus content to be brought about, as far as possible, by selection of the components of the ration, but the differences in the quantity of ingested phosphorus to be intensified both by special treatment of the ration and by adding to it particular phosphorus-bearing bodies, the effect of which it is desired to study.

2. Modification of the phosphorus content of the ration by variations, as far as possible, in the amount present of a particular phosphorus-bearing body.

3. The changes from a high to a low phosphorus ration to be both abrupt and gradual.

4. The maintenance of the rations on the same nutritive plane, excepting in the variations of the phosphorus content.

5. The feeding of weighed and carefully sampled rations, and the collection and weighing of the excreta and product, to be accompanied by such chemical determinations as are essential to the solution of the problem in hand.

It was expected that this plan of work would furnish data on the following points:

1. The amounts and kinds of phosphorus-bearing bodies in certain cattle foods (already published).

2. The transformations and distribution of phosphorus-bearing bodies brought about by the metabolic changes in the animal.

3. The influence of the supply of phosphorus compounds, both in kind and in quantity, upon the physiological status of the animal, and upon the composition and yield of product, whether milk or otherwise.

The rations.—The first matter requiring attention in the experiment was the selection or preparation of foods containing small amounts of phosphorus. It was found that rice, wheat gluten, and oat straw were as low in phosphorus as any feeds that could be selected and these, combined with the washed wheat bran, constituted the rations low in phosphorus.

The extraction of phytin from the wheat bran was easily accomplished. The bran was allowed to soak over night in warm water, a slight acid fermentation being induced. When the water showed a slight acidity to litmus, the bran was placed in sacks and thoroughly leached with more water. After being air-dried it was ready for use.

The high phosphorus rations were made up from oat straw, hominy, or rice meal, whole wheat bran and wheat gluten. Through

the use of the wheat gluten which contained from 70 to 75 per cent of protein, it was easy to regulate the protein supply so as to make it fairly uniform in the rations compared.

The management of the experiments. — The animals under observation stood by themselves in a warmed room especially arranged for experimental work. They were fed from a metal-lined box which allowed the attendant to recover all uneaten food. The daily ration was given in three equal portions, morning, noon, and night. Water was offered at stated times and the animal was weighed at the same hour each day. Two men were employed for the collection of the excreta, one during the night and the other during the day. The excreta were caught in tin vessels, the one used for the urine being so constructed as to prevent loss by spattering. There was a loss of urine in but a single instance and that was small. The weights of urine and fæces recorded represent that which was voided during twenty-four hours, from six o'clock A.M. The animal was milked four times a day at as nearly uniform times as practicable, viz., seven A.M., noon, five P.M., and midnight.

Method of sampling and analysis. — The rations were weighed out at several different times during the course of the experiment and each time this was done samples were taken of the various feeds. The similarity in composition of these several portions indicates that the mixing and sampling were thoroughly accomplished. The milk, urine, and fæces were taken directly to the laboratory and immediately weighed and sampled, excepting that the noon and night's milk was kept in an ice box until morning, when the product for an entire day was thoroughly mixed together and sampled. Reserve samples of milk and urine were always kept, fermentative changes in these being prevented by the use of formaldehyde and toluol. The fæces were thoroughly mixed by prolonged stirring and five pound samples of the fresh material were taken for drying and for nitrogen determinations. These samples were dried over steam coils at a temperature not exceeding 60° C.

In general the analytical methods of the A.O.A.C. were followed. During some of the time the fat in the milk was estimated both by the Babcock and ether extraction methods. Neumann's method was used in all the total phosphorus determinations. For the separation of inorganic from organic phosphorus the method described in Bulletin No.238 of this Station was employed. This method also gave an opportunity to estimate what we have called "fixed" phosphorus

and which probably quite closely represents the phosphorus of the true nucleo-proteids and nucleins. In the urine only total phosphorus was determined, as attempts to separate inorganic from organic forms, even if the latter existed in appreciable quantities, were futile.

The experimental animals. — The animal (cow 1) selected for use in our first experiment was a vigorous grade Holstein. Her weight was approximately 1100 pounds. When the experiment began she was in fair flesh and about three months advanced in the period of lactation. She possessed a vigorous appetite, a characteristic which we regarded as essential to the entire consumption of the ration containing the washed bran because of its probable lack of palatableness.

The animal (cow 2) used in the second and third experiments was also a vigorous grade Holstein, weighing 966 pounds and in good condition when placed in the experiment, and was from one to two months advanced in the period of lactation. She manifested a good appetite throughout the experiments and consumed daily all the food offered. Her health remained apparently unimpaired by the food and treatment which she received.

Numerical Results of the Experiments.

Extensive data were necessarily recorded in experiments involving such numerous weighings and analyses during periods of two months or more in each experiment. The figures herewith presented are confined to those which are essential to a critical analysis of our conclusions.

The tables are made up either of daily records or of averages for certain periods and cover those times when it was believed that the animals had adjusted themselves to the ration being fed. In some cases the effects of the transition periods are shown.

Experiment 1 (1904). — This was a comparison of the effect of rations differing greatly in their phosphorus content, especially in phytin phosphorus. The essential differences in the two rations compared in this experiment were brought about by substituting washed bran for the unwashed.

The character and sequence of the rations in the first phytin experiment. — *Ration 1.* — The low phosphorus ration was fed from noon, March 8, to the morning of March 17. The animal had been slowly brought to this ration by preliminary feeding for nine days previous to March 8.

Transition period. — A gradual change from a low phosphorus ration to one high in phosphorus, occupying from noon, March 17, to the morning of April 5. During this period the washed bran was decreased one-half pound daily, the same amount of the whole bran being substituted. The rice was decreased by about one-third of a pound per day, and the hominy feed increased one-fourth of a pound. The wheat gluten was decreased 12 gm., or about one-fortieth of a pound daily.

Ration 2. — The high phosphorus ration was fed from noon, April 5, to the morning of April 19. This ration differed from Ration 1 in that its content of phytin phosphorus was approximately twenty times as large.

Ration 1. — Sudden change from a high phosphorus ration to one low in phosphorus, the latter being fed from noon, April 19, to the morning of May 2. This ration was eaten satisfactorily until the morning of April 24, when it was refused. The animal became seriously constipated, and, as compaction was feared, one-half pound of Epsom salts was administered with negative results. On April 25, one and one-fourth pounds of Epsom salts was given and the ration withheld at noon. This produced the desired result, and the animal began to eat again on the 26th, only part of the ration being given. The amount fed was increased until, on the 28th of April, the cow was receiving her full ration (Ration 1) and appeared perfectly normal.

Ration 2. — Sudden change from the low phosphorus ration to the one high in phosphorus. Ration 2 fed from noon, May 2, to the morning of May 15.

Transition period. — From noon, May 15, to noon, May 20. During this period the unwashed bran was decreased two pounds daily, and the washed bran substituted by the same amount. The hominy feed was decreased one pound daily, and the rice increased one and one-half pounds daily. The wheat gluten was increased about one-fifth of a pound daily. Marked dryness of the fæces accompanied this transition, but the animal continued to consume all the ration.

Ration 1. — The low phosphorus ration was fed from noon, May 20, to the morning of June 17.

Ration 2. — Sudden change from a low phosphorus to a high phosphorus ration, the latter being fed from noon, June 17, to the morning of June 24.

This experiment covered one hundred and eight days. The collection and analysis of the urine and fæces covered seventy-one days, this being done continuously during fifty-four days. A complete analysis of the milk was made daily.

TABLE I.

THE RATIONS FED.

Ingredients.	Ration 1.	Ingredients.	Ration 2.
Oat straw	10 lbs.	Oat straw	10 lbs.
Washed bran . . .	10 "	Whole bran . . .	10 "
Rice	6 "	Hominy	5 "
Wheat gluten . .	1½ "	Wheat gluten . .	1 "
Total	27½ lbs.	Total	26 lbs.

TABLE II.

THE COMPOSITION OF THE FEEDING STUFFS USED.

Lab. No.	Feeds.	Water.	Protein.	Fat.	Phosphorus.		
					Total.	Soluble.	Inorganic.
		per cent	per cent	per cent	per cent	per cent	per cent
1	Oat straw . .	18.29	2.34	1.87	0.151	0.082	0.058
7	Wheat gluten	7.71	72.12	1.39	0.201	0.056	0.000
3	Rice	14.05	8.37	0.33	0.076	0.011	0.000
8	Washed bran	7.23	11.19	4.07	0.099	0.013	0.000
2	Whole bran .	12.13	14.50	4.42	1.32	0.98	0.000
38	Hominy . .	14.41	9.25	5.81	0.457	0.27	0.000

TABLE III.

QUANTITIES AND PERCENTAGES OF DIGESTIBLE NITROGEN AND DRY MATTER FED.

Date.	P fed.	Dry matter daily.			Nitrogen daily.		
		Fed.	In fæces.	Digested.	Fed.	In fæces.	Digested.
	grams	grams	grams	per cent	grams	grams	per cent
March 10–16 .	12.8	8908	2872	67.7	178	52	71.6
April 12–18 . .	78.7	10039	3782	62.3	210	59	71.7
April 28–May 1	16.0	10863	4471	58.8	215	79	63.3
May 9–15 . .	83.3	10214	3993	61.0	215	64	70.1
May 22–26 . .	21.4	11335	4018	64.5	216	70	67.4
June 10–16 . .	15.1	11354	4537	60.0	224	82	63.3

TABLE IV.

PERCENTAGE COMPOSITION OF THE MILK WITH BOTH HIGH AND LOW PHOSPHORUS INGESTION, INCLUDING A TRANSITION PERIOD.

Date.	P fed daily.	In milk.					Date.	P fed daily.	In milk.				
		Proteids.	Casein.	Fat.	Sugar.	Total solids.			Proteids.	Casein.	Fat.	Sugar.	Total solids.
	grams	p. c.	p. c.	p. c.	p. c.	p. c.		grams	p. c.	p. c.	p. c.	p. c.	p. c.
May 9	83.0	2.65	2.25	3.25	5.64	11.54	May 22	21.0	2.66	2.16	2.20	5.91	10.67
10	83.0	2.64	2.13	3.50	5.68	11.82	23	21.0	2.67	2.23	2.15	6.04	10.91
11	83.0	2.68	2.21	3.40	5.70	11.78	24	21.0	2.66	2.20	2.25	6.00	10.81
12	83.0	2.46	2.00	3.35	5.61	11.42	25	21.0	2.61	2.12	2.30	5.96	10.82
13	83.0	2.62	2.15	3.35	5.72	11.69	26	21.0	2.62	2.19	2.45	5.84	10.76
14	83.0	2.61	2.22	3.20	5.55	11.56	27	21.0	2.69	2.29	2.60	5.79	10.93
15	83.0	2.69	2.25	3.05	5.34	11.23	28	15.6	2.70	2.26	2.60	5.69	10.99
16	69.0	2.68	2.18	2.85	5.83	11.56	29	15.6	2.70	2.34	2.70	5.80	11.10
17	55.0	2.60	2.17	2.40	6.09	11.54	30	15.6	2.63	2.36	2.80	5.85	11.18
18	36.0	2.59	2.20	2.25	5.91	11.35	31	15.6	2.73	2.34	2.90	5.83	11.36
19	29.0	2.59	2.18	2.20	6.07	11.06	June 1	15.6	2.70	2.29	2.80	5.87	11.47
20	21.0	2.65	2.25	2.10	5.97	10.87	2	15.6	2.55	2.16	2.70	5.93	11.28
21	21.0	2.62	2.18	2.20	5.91	10.73							

TABLE V.

PERCENTAGES OF VARIOUS FORMS OF PHOSPHORUS IN THE MILK.

Date.	P fed daily.	In milk.			Date.	P fed daily.	In milk.		
		Total.	Soluble.	Inorganic.			Total.	Soluble.	Inorganic.
	grams	per cent	per cent	per cent		grams	per cent	per cent	per cent
May 9	83.0	0.082	0.071	0.063	May 22	21.0	0.094	0.071	0.069
10	83.0	0.078	0.068	0.060	23	21.0	0.091	0.074	0.072
11	83.0	0.083	0.066	0.056	24	21.0	0.092	0.075	0.072
12	83.0	0.076	0.065	0.059	25	21.0	0.093	0.074	0.074
13	83.0	0.083	0.065	0.061	26	21.0	0.093	0.071	0.073
14	83.0	0.083	0.070	0.065	27	21.0	0.090	0.070	0.070
15	83.0	0.084	0.064	0.062	28	15.6	0.090	0.077	0.072
16	69.0	0.083	0.067	0.065	29	15.6	0.091	0.072	0.076
17	55.0	0.084	0.066	0.063	30	15.6	0.089	0.070	0.072
18	36.0	0.086	0.068	0.066	31	15.6	0.092	0.073	0.076
19	29.0	0.079	0.070	0.063	June 1	15.6	0.092	0.072	0.060
20	21.0	0.080	0.066	0.065	2	15.6	0.079	0.075	0.065
21	21.0	0.093	0.067	0.065					

TABLE VI.

TOTAL PHOSPHORUS BALANCES.

Date.	Daily income and outgo of phosphorus.				
	Fed.	Total outgo.	In fæces.	In milk.	In urine.
	grams	grams	grams	grams	grams
March 10–16 . .	12.8	22.7	8.40	14.2	0.10
April 12–18 . .	78.7	70.6	55.70	11.0	3.90
April 28–May 1 .	16.0	23.8	13.29	10.1	0.37
May 9–15 . . .	83.3	70.4	56.70	8.8	4.88
May 22–26. . .	21.4	22.7	9.59	13.0	0.11
June 18–24 . .	15.1	20.5	9.40	11.0	0.12

TABLE VII.

DISTRIBUTION AND BALANCES OF NUCLEO-PROTEID PHOSPHORUS.

Date.	Total P fed daily.	Daily income and outgo fixed phosphorus.				
		Fed.	Total outgo.	In fæces.	In milk.	In urine.
	grams	grams	grams	grams	grams	grams
March 10–16 . .	12.8	7.6	6.6	3.9	2.6	0.00
April 12–18 . . .	78.7	24.4	9.5	7.6	1.9	0.00
April 28–May 1 .	16.0	9.0	8.7	6.9	1.8	0.00
May 9–15 . . .	83.3	28.6	7.5	5.8	1.7	0.00
May 22–26 . . .	21.4	14.2	7.7	5.2	2.4	0.00
June 18–24 . . .	15.1	7.5	7.8	5.2	2.6	0.00

TABLE VIII.

DISTRIBUTION AND BALANCES OF SOLUBLE ORGANIC PHOSPHORUS.

Date.	Total P fed daily.	Daily income and outgo soluble organic phosphorus.				
		Fed.	Total outgo.	In fæces.	In milk.	In urine.
	grams	grams	grams	grams	grams	grams
March 10–16 . .	12.8	2.5	2.0	1.80	0.37	0.00
April 12–18 . . .	78.7	51.1	4.1	3.90	0.16	0.00
April 28–May 1 .	16.0	4.3	2.0	1.40	0.56	0.00
May 9–15 . . .	83.3	52.1	6.2	5.30	0.95	0.00
May 22–26 . . .	21.4	3.7	2.2	1.70	0.52	0.00
June 18–24 . . .	15.1	4.1	1.6	0.98	0.64	0.00

TABLE IX.

DISTRIBUTION AND BALANCES OF INORGANIC PHOSPHORUS.

Date.	Total P fed daily.	Daily income and outgo inorganic phosphorus.				
		Fed.	Total outgo.	In fæces.	In milk.	In urine.
	grams	grams	grams	grams	grams	grams
March 10–16 . .	12.8	2.63	14.2	2.8	11.3	0.10
April 12–18. . .	78.7	2.60	56.6	44.1	8.6	3.90
April 28–May 1 .	16.0	2.60	13.1	4.9	7.9	0.37
May 9–15 . . .	83.3	2.60	57.2	45.6	6.7	4.90
May 22–26 . . .	21.4	3.50	12.7	2.6	10.0	0.11
June 18–24 . . .	15.1	3.40	11.9	3.2	8.6	0.12

TABLE X.

RELATION BETWEEN NITROGEN AND PHOSPHORUS OUTGO.

Date.	Nitrogen.		Daily income and outgo of phosphorus.					
	Fed daily.	Outgo daily.	Fed.	Soluble[1] fed.	Inorganic fed.	Total excreted.	Soluble[1] excreted.	Inorganic excreted.
	grams	grams	grams	grams	grams	grams	grams	grams
March 10–16 . .	178	190	12.8	5.18	2.6	22.7	16.2	14.2
April 12–18 . .	209	205	78.7	53.70	2.6	70.6	60.8	56.7
April 28–May 1 .	215	215	16.0	6.90	2.6	23.8	15.2	13.1
May 9–15 . . .	215	208	83.3	54.7	2.6	70.4	62.9	57.1
May 22–26 . .	216	200	21.4	7.27	3.5	22.7	14.9	12.7

[1] The soluble includes the inorganic.

TABLE XI.

EFFECTS OF THE INGESTION OF PHOSPHORUS COMPOUNDS UPON THE COMPOSITION OF THE MILK, SHOWN BY PERIODS.

Date.	Total P fed.	In milk.				
		Proteids.	Casein.	Fat.	Sugar.	Total solids.
	grams	per cent	per cent	per cent	per cent	per cent
March 10–16 . .	12.8	2.52	2.08	3.26	5.59	11.37
April 12–18 . .	78.7	2.55	2.12	3.11	5.62	11.28
April 28–May 1 .	16.0	2.58	2.16	2.55	5.65	10.78
May 9–15 . . .	83.3	2.61	2.15	3.37	5.65	11.65
May 22–26 . .	21.4	2.64	2.18	2.20	5.95	10.80
June 10–16 . .	15.1	2.77	2.31	2.93	5.64	11.30
June 18–24 . .	80.7	2.73	2.31	3.27	5.48	11.47

TABLE XII.

EFFECTS OF THE INGESTION OF PHOSPHORUS COMPOUNDS UPON THE YIELD OF MILK AND MILK SOLIDS, AND UPON THE EXCRETION OF URINE.

Date.	P fed daily.	Yield milk daily.	Daily yield milk solids.			Weight urine daily.
			Casein nitrogen.	Fat.	Total solids.	
	grams	grams	grams	grams	grams	grams
March 10–16 .	12.8	16335	53.1	531	1856	4974
April 12–18 . .	78.7	12813	42.8	400	1454	10555
April 28–May 1 .	16.0	12027	40.8	306	1296	9560
May 9–15 . . .	83.3	11018	37.5	368	1276	12723
May 22–26 . .	21.4	13833	47.4	305	1495	10147
June 10–16 . .	15.1	12043	43.6	350	1359	....
June 18–24 . .	80.7	10428	37.8	341	1204	....

Discussion of results of Experiment I. — The following is a somewhat concise statement of the main results of the foregoing experiment, a fuller discussion being reserved until after a consideration of the outcome of further experiments:

1. *The comparative nutritive value of the rations.* — It is shown by the figures of Table III, that outside of the changes brought about by washing the bran there was nothing in the kind, quantities, or variations in the nutrients fed that could account for the different effect of the two rations. The digestible dry matter consumed varied from 13.3 pounds to 16.1 pounds daily, of which from 2.6 pounds to 2.9 pounds consisted of digestible protein. In no period was the ration so scanty as to produce abnormal results and the changes in the quantity of digestible dry matter and protein eaten did not especially favor either ration.

2. *The amounts and forms of ingested phosphorus in the two rations.* — The amounts of phosphorus ingested in the several periods varied greatly, ranging from 12.8 gm. to 21.4 gm. daily in the low phosphorus period, the daily quantities in the three high phosphorus periods being 78.7 gm., 83.3 gm., and 80.7 gm. These differences were brought about chiefly by removing the phytin from the bran, 62 per cent to 65 per cent of the phosphorus supply in ration two or from 51 to 52 gm. daily being carried in this compound. The daily amount of fixed phosphorus in ration two varied from 24.4 to 28.6 gm., or from 31 per cent to 34 per cent of the whole. The quantity of proteid phosphorus in this ration was two or three times greater than that of ration one, because of the fact that the unwashed bran and hominy feed contained much more phosphorus in insoluble forms than did the washed bran and rice. The amount of inorganic phosphorus fed, which was found entirely in the straw, was very small and was uniform in quantity. It is clear, therefore, that in studying the comparative effect of these two rations as far as phosphorus compounds are concerned, our attention should be fixed mainly upon phytin and what we have designated as the fixed phosphorus compounds or what doubtless approximates very closely to the nucleo-proteids and nucleins. (See Tables VI and VII.)

3. *The relation in amounts and forms of the ingested and the excreted phosphorus.* — The amount of outgoing phosphorus followed in a general way the quantity ingested, but within narrower limits. It is interesting to note that the animal excreted considerably more phosphorus than she received during the time that the washed bran ration was being fed. The fact is worthy of record that she was maintained in an apparently healthy condition on a ration which supplied 21.2 gm. of phosphorus daily from May 20 to May 26, and only 15.6 gm. daily from May 26 to June 16, and at the end of the

twenty-seven days she was found to be losing an average of 26 gm. per day.

On the other hand, when the supply of phosphorus was liberal (ration two) there was a storage of that element in the body of the cow, amounting in one period to an average of 8 gm. daily, while in another the average was 13 gm.

The proportionate distribution of the outgoing phosphorus among its various compounds was quite unlike what was found in the food. Through catabolic changes all, or nearly all, the phosphorus of the phytin and of the unused digested proteids was reduced to inorganic forms which were excreted. The variations in the quantities of the outgoing phosphorus in the several periods were found, therefore, to consist almost wholly of changes in the proportions of inorganic salts of this element in the egesta. The data furnish no evidence that any synthesis of the phosphorus-bearing proteids occurred. The quantities of these latter bodies in the milk and egesta appear in no case to be materially greater than the food supply. (See Tables VI–IX.)

4. *Distribution of outgoing phosphorus compounds in the milk and egesta.* — Variations in the amount of outgoing phosphorus, corresponding to the rise and fall of phosphorus compounds in the rations, took place chiefly in the fæces, though to a small extent in the urine. Moreover, as previously indicated, this increase or decrease in the phosphorus bodies of the egesta was found to occur almost wholly with the inorganic salts. The percentage of these salts in the milk suffered no appreciable change. The proportions of organic phosphorus bodies remained fairly constant in the milk and egesta in spite of marked changes in the quantities of the ingested phosphorus compounds. It is especially noteworthy that the secretion of casein, the characteristic nucleo-proteid of milk, seemed to be in no way affected by the food supply of similar bodies or by variations in the phosphorus supply in other forms of combination, even when there was a phosphorus deficiency during a long period. (See Tables VI–IX.)

5. *Physiological effects due to variations in the phosphorus bodies of the two rations.* — No evidence appears that the increase or decrease of the phosphorus compounds in the ration influenced either the digestibility of the total dry matter or of the nitrogen compounds, neither was the storage or excretion of nitrogen apparently affected. No relation was found to exist between the nitrogen and phosphorus balances. Certain well defined and marked effects were observed, however. (See Tables III and X.)

In our attempts at transition from one ration to the other we soon learned that it was not possible to change the cow suddenly from a ration rich in phytin to one low in this compound and keep the animal in normal health. Such a change caused her to become seriously constipated and recourse to a purgative was necessary. When the change from the unwashed bran to the washed bran ration was made slowly the animal gave no evidence of serious disturbance of health and appetite but the fæces always became much darker in color and much dryer.

The removal of the phytin from the bran had the effect of materially diminishing the volume of urine. Marked changes in the amount and character of the milk secreted were also observed. The transition from the high phytin to the low phytin ration caused an increase in the volume of milk accompanied by very large decrease in its percentage of fat. As shown in Table IV, the maximum decrease in fat amounted to 1.4 per cent and kept pace with the withdrawal of phytin from the ration. The total solids decreased about 0.8 per cent. Table IV also shows that the maximum decrease in the percentage of fat was not permanently maintained but that even during the feeding of the low phosphorus ration there was a slow restoration of the proportion of fat, although the percentage did not rise to as high a point as was found during the feeding of the high phytin ration. (See Tables IV, XI, and XII.)

6. *Influence of the two rations upon production.* — Although the increase in milk flow tended to counteract the decreased percentage of fat, nevertheless the total fat production in a given length of time was considerably lessened by the substitution of washed bran for the unwashed. Similar results did not obtain with the total solids and casein. (See Table XII.)

7. *Œstrum period.* — It is a very suggestive fact that the œstrum appeared with this animal for the last time on June 12, a disturbance which followed a long-continued feeding of the washed bran ration. Whether the cessation of this period was in any way connected with the removal of the phosphorus and its associated elements from the bran can be at present only a matter of theory.

Experiment 2 (1904–5). — This was a comparison of rations carrying unlike quantities of nucleo-phosphorus, with minor differences in their phytin content. As stated, our original plan involved mainly a study of the influence on milk secretion of rations, rich and poor, in nucleo-phosphorus.

This second experiment was planned, therefore, with especial reference to this phase of our general investigation. Its general procedure was very similar to that followed in the first experiment, viz., the feeding of two rations in different periods which differed in an emphatic way in their content of a particular class of phosphorus-bearing bodies. In this instance the variation was made as far as possible with those phosphorus compounds insoluble in dilute hydrochloric acid, consisting mainly of the nucleo-proteids and nucleins.

Variations in the supply of nucleo-phosphorus in the rations were accomplished in the following manner: A basal ration was compounded consisting of rice, washed bran, and oat straw. To this was added germ oil meal, in order to increase the supply of nucleo-phosphorus. This meal was found to be especially rich in forms of phosphorus insoluble in dilute hydrochloric acid. An estimation of the purin bases, after cleavage with dilute hydrochloric acid, showed this material to be rich in nucleins, assuming, of course, that these bases can only originate from nucleo structures. The ration poor in nucleo-phosphorus was compounded by withdrawing the germ oil meal and substituting for it wheat gluten and rice meal. It was expected that the amounts of digestible dry matter and protein would be quite similar in the two rations.

Character and sequence of the rations. — During a preliminary period Ration 1 was fed for several days, in order to adjust the animal to its influences before observations were taken.

Ration 1. — Maximum amount of nucleo-phosphorus. Observations with this ration were begun at noon, December 20, this period continuing to the morning of January 3.

Transition period. — From noon, January 3, to the morning of January 6. During this period the germ oil meal was reduced one and one-half pounds daily, and the rice was increased one pound daily. One-half pound of wheat gluten was added each day.

Ration 2. — Minimum amount of nucleo-phosphorus. This ration was fed from noon, January 6, to the morning of January 19.

Ration 1. — A sudden change was made from Ration 2 to Ration 1, and the latter was fed from noon, July 19, to the morning of February 2.

Ration 2. — A sudden change was made from Ration 1 to Ration 2, and the latter was fed from noon, February 2, to the morning of February 16.

The period of observation in this experiment covered fifty-eight days, during which time there was a continuous collection and analysis of the milk and excreta.

TABLE XIII.

THE RATIONS FED.

Ingredients.	Ration 1.	Ingredients.	Ration 2.
Oat straw	10 lbs.	Oat straw	10 lbs.
Washed bran . .	10 "	Washed bran . .	10 "
Corn germ meal .	6 "	Wheat gluten . .	2 "
Rice meal	3 "	Rice meal	7 "
Total	29 lbs.	Total	29 lbs.

TABLE XIV.

THE COMPOSITION OF THE FEEDING STUFFS USED.

Feeds.	Water.	Protein.	Fat.	Phosphorus.		
				Total.	Soluble.	Inorganic
	per cent	per cent	per cent	per cent	per cent	per cent
Oat straw . . .	9.76	4.25	1.87	0.156	0.127	0.102
Washed bran . .	8.40	10.93	4.13	0.145	0.054	0.000
Rice meal . . .	13.04	7.25	0.33	0.074	0.006	0.000
Corn germ meal .	8.26	22.50	8.70	0.817	0.271	0.000
Wheat gluten . .	6.84	72.80	1.44	0.073	0.008	0.000

TABLE XV.

QUANTITIES AND PERCENTAGES OF DIGESTIBLE NITROGEN AND DRY MATTER FED.

Date.	P fed daily.	Dry matter daily.			Nitrogen daily.		
		Fed.	In fæces.	Digested.	Fed.	In fæces.	Digested.
	grams	grams	grams	per cent	grams	grams	per cent
Dec. 27–Jan. 2	37	12056	4917	59.2	226.1	93.2	58.7
Jan. 13–19 . .	18	11906	4236	64.4	270.5	83.5	69.1
Jan. 27–Feb. 2	37	11967	4676	60.9	229.9	87.4	61.9
Feb. 10–16. .	20	11831	4408	62.2	289.0	84.6	70.7

TABLE XVI.

PERCENTAGE DAILY COMPOSITION OF THE MILK WITH BOTH HIGH AND LOW PHOSPHORUS INGESTION, INCLUDING TWO TRANSITION PERIODS.

Date.	P fed daily.	In milk.					Date.	P fed daily.	In milk.				
		Proteids	Casein	Fat.	Sugar.	Total solids.			Proteids	Casein	Fat.	Sugar.	Total solids.
	grams	p. c.	p. c.	p. c.	p. c.	p. c.		grams	p. c.	p. c.	p. c.	p. c.	p. c.
Dec. 30	37	2.56	2.13	2.70	5.47	10.73	Jan. 30	37	2.70	2.23	2.80	5.68	11.18
31	37	2.55	2.06	2.85	5.52	10.92	31	37	2.65	2.25	2.75	5.81	11.21
Jan. 1	37	2.53	2.06	2.85	5.51	10.89	Feb. 1	37	2.75	2.23	2.75	5.68	11.18
2	37	2.53	2.08	2.80	5.51	10.84	2	37	2.64	2.21	2.65	5.72	11.01
3	32	2.53	2.03	2.80	5.56	10.89	3	18	2.74	2.27	2.70	5.69	11.13
4	27	2.58	2.06	3.15	5.51	11.24	4	18	2.80	2.34	2.65	5.61	11.06
5	23	2.63	2.18	2.65	5.44	10.72	5	20	2.82	2.30	2.60	5.60	11.02
6	18	2.77	2.18	2.70	5.36	10.83	6	20	2.76	2.21	2.70	5.51	10.97
7	18	2.65	2.10	2.40	5.67	10.72	7	20	2.77	2.23	2.55	5.43	10.75
8	18	2.75	2.12	2.50	5.44	10.69	8	20	2.74	2.22	2.40	5.56	10.70
9	18	2.65	2.13	2.55	5.67	10.87	9	20	2.77	2.27	2.40	5.68	10.85
10	18	2.73	2.18	2.45	5.67	10.85	10	20	2.80	2.25	2.45	5.53	10.78
11	18	2.69	2.15	2.70	5.52	10.91	11	20	2.81	2.27	2.60	5.37	10.87
12	18	2.73	2.16	2.75	5.47	10.95	12	20	2.79	2.22	2.65	5.73	11.17

TABLE XVII.

PERCENTAGES OF VARIOUS FORMS OF PHOSPHORUS IN THE MILK.

Date.	P fed daily.	In milk.			Date.	P fed daily.	In milk.		
		Total.	Soluble.	Inorganic.			Total.	Soluble.	Inorganic.
	grams	per cent	per cent	per cent		grams	per cent	per cent	per cent
Dec. 30	37	0.102	0.083	0.072	Jan. 6	18	0.109	0.086	0.071
31	37	0.104	0.084	0.071	7	18	0.106	0.084	0.074
Jan. 1	37	0.103	0.083	0.065	8	18	0.104	0.085	0.074
2	37	0.102	0.080	0.071	9	18	0.104	0.086	0.078
3	32	0.100	0.084	0.069	10	18	0.104	0.083	0.078
4	27	0.103	0.081	0.067	11	18	0.104	0.082	0.069
5	23	0.104	0.081	0.068	12	18	0.107	0.087	0.073

TABLE XVIII.

TOTAL PHOSPHORUS BALANCES.

Date.	Daily income and outgo of phosphorus.				
	Fed.	Total outgo.	In fæces.	In milk.	In urine.
	grams	grams	grams	grams	grams
Dec. 27–Jan. 2 .	37	42.0	24.4	17.5	0.12
Jan. 13–19 . . .	18	30.2	11.6	18.5	0.09
Jan. 27–Feb. 2 .	37	39.8	22.1	17.6	0.07
Feb. 10–16 . .	20	29.2	10.9	18.2	0.07

TABLE XIX.

DISTRIBUTION AND BALANCES OF NUCLEO-PHOSPHORUS.

Date.	Total P fed daily.	Daily income and outgo fixed phosphorus.				
		Fed.	Total outgo.	In fæces.	In milk.	In urine.
	grams	grams	grams	grams	grams	grams
Dec. 27–Jan. 2 .	37	21.5	11.4	8.0	3.4	0.00
Jan. 13–19 . . .	18	9.5	9.7	6.1	3.6	0.00
Jan. 27–Feb. 2 .	37	21.2	11.4	7.9	3.5	0.00
Feb. 10–16 . . .	20	11.4	10.5	6.8	3.7	0.00

TABLE XX.

DISTRIBUTION AND BALANCES OF SOLUBLE ORGANIC PHOSPHORUS COMPOUNDS.

Date.	Total P fed daily.	Daily income and outgo soluble organic phosphorus.				
		Fed.	Total outgo.	In fæces.	In milk.	In urine.
	grams	grams	grams	grams	grams	grams
Dec. 27–Jan. 2 .	37	11.1	3.3	1.4	1.9	0.00
Jan. 13–19 . . .	18	4.1	3.2	1.4	1.8	0.00
Jan. 27–Feb. 2 .	37	11.2	3.2	1.5	1.7	0.00
Feb. 10–16 . . .	20	4.8	2.9	0.7	2.2	0.00

TABLE XXI.

DISTRIBUTION AND BALANCES OF INORGANIC PHOSPHORUS.

Date.	Total P fed daily.	Daily income and outgo inorganic phosphorus.				
		Fed.	Total outgo.	In fæces.	In milk.	In urine.
	grams	grams	grams	grams	grams	grams
Dec. 27–Jan. 2 .	37	4.7	27.3	15.0	12.1	0.12
Jan. 13–19 . . .	18	4.7	17.3	4.1	13.1	0.09
Jan. 27–Feb. 2 .	37	4.7	25.3	12.7	12.5	0.07
Feb. 10–16 . . .	20	4.7	15.8	3.4	12.3	0.07

TABLE XXII.

RELATION BETWEEN NITROGEN AND PHOSPHORUS EXCRETION.

Date.	Nitrogen.		Daily income and outgo of phosphorus.					
	Fed daily.	Outgo daily.	Fed.	Soluble fed.	Inorganic fed.	Total excreted.	Soluble[1] excreted.	Inorganic excreted.
	grams	grams	grams	grams	grams	grams	grams	grams
Dec. 27–Jan. 2 .	226	217	37.3	15.8	4.7	42.0	30.4	27.2
Jan. 13–19 . .	270	266	18.2	8.8	4.7	30.2	20.3	17.2
Jan. 27–Feb. 2 .	229	212	37.3	15.8	4.7	39.8	28.4	27.2
Feb. 10–16	279	263	20.2	8.8	4.4	29.2	18.6	15.7

[1] The soluble includes the inorganic.

TABLE XXIII.

EFFECTS OF THE INGESTION OF PHOSPHORUS COMPOUNDS UPON THE COMPOSITION OF THE MILK.

Date.	Total P fed daily.	In milk.				
		Proteids.	Casein.	Fat.	Sugar.	Solids.
	grams	per cent	per cent	per cent	per cent	per cent
Dec. 27–Jan. 2 .	37	2.58	2.09	2.78	5.47	10.87
Jan. 13–19 . .	18	2.77	2.19	2.74	5.49	11.00
Jan. 27–Feb. 2 .	37	2.66	2.21	2.83	5.67	11.08
Feb. 10–16 . .	20	2.81	2.29	2.67	5.49	10.97

TABLE XXIV.

EFFECTS OF THE INGESTION OF PHOSPHORUS COMPOUNDS UPON THE YIELD OF MILK AND MILK SOLIDS AND UPON THE EXCRETION OF URINE.

Date.	P fed daily.	Yield milk daily.	Daily yield milk solids.			Weight urine daily.
			Casein nitrogen.	Fat.	Total solids.	
	grams	grams	grams	grams	grams	grams
Dec. 27–Jan. 2 .	37	16715	55	464	1812	3600
Jan. 13–19 . . .	18	17620	61	482	1935	5752
Jan. 27–Feb. 2 . .	37	16768	58	462	1858	4528
Feb. 10–16 . . .	20	16960	61	452	1860	5468

Discussion of results. — The data furnished by this experiment appear to justify the following statements:

1. *The comparative nutritive value of the rations.* — The quantity of digestible dry matter fed daily varied in the several periods from 15.7 pounds to 16.9 pounds, the amounts ingested in the two high phosphorus periods being not greatly unlike what was eaten in the two low phosphorus periods. The quantities of digestible protein consumed in the several periods varied from 1.8 pounds daily to 2.8

pounds. The low phosphorus ration carried in all cases more digestible protein than did the other ration. The supply of nutrients was abundant at all periods. (See Table XIII.)

2. *The amounts and forms of ingested phosphorus in the two rations.* — The quantities of phosphorus compounds which the two rations carried were not so greatly unlike as was the case in the first experiment. In both periods of the higher phosphorus feeding the amount of ingested phosphorus was 37.3 gm., while in the other two periods the quantity was 18.2 gm. and 20.2 gm. respectively. The proportions of the various classes of phosphorus compounds in the two rations were quite unlike what was found in the first experiment. Approximately 57 per cent existed in forms insoluble in dilute hydrochloric acid or what we believe to be chiefly nucleins and nucleoproteids. Thirty per cent existed as soluble organic phosphorus in Ration 1, and from 23 to 24 per cent in Ration 2. The inorganic phosphorus remained practically a constant quantity in the two rations, viz., 4.7 gm. daily. This experiment was also unlike the first one in that the two rations differed chiefly in the quantities of insoluble phosphorus compounds which they carried rather than in the proportions of phytin. (See Tables XVIII–XXI.)

3. *The relation in amounts and forms of the ingested and the outgoing phosphorus.* — In no period was the quantity of ingested phosphorus equal to the outgoing amount. With Ration 1 this deficiency was from 2.5 to 4.7 gm. daily, while with Ration 2 it was 12 gm. in one period and 9 gm. in the other. These data furnish another illustration of the fact that a cow is able to make good a phosphorus deficiency in the ration from the store contained in her body. We regard it as unfortunate that in this experiment we were not able to feed an excess of phosphorus compounds in the high phosphorus ration.

In regard to the proportionate distribution of phosphorus compounds in the ingesta as compared with the egesta, the facts are essentially similar to what was found in the first experiment. The quantity of excreted phosphorus increased and decreased with the amount fed, and as in the first experiment the variations in the outgoing amount were found to occur chiefly with the proportions of inorganic forms in the egesta. (See Tables XVIII–XXI.)

4. *Distribution of outgoing phosphorus compounds in the milk and egesta.* — The variations of the outgoing phosphorus are again found to occur almost wholly in the fæces through a rise or fall of inorganic

forms. This second experiment furnishes no evidence of changes in the distribution of other forms of phosphorus in the milk and egesta, due to variations in the quantities of phosphorus compounds in the ration. (See Tables XIX–XXI.)

5. *Physiological effects due to variations in the phosphorus bodies of the two rations.* — There is a marked difference between this experiment and the first one in the extent, at least, of the physiological disturbances observed. The results are alike in that the amount of phosphorus excretion had no effect upon the storage or excretion of nitrogen. The results are unlike in the influence of changing rations upon the health of the animal and the condition of the fæces. Nothing like constipation was observed, nor was there any marked change in the condition or color of the fæcal discharge.

There was a perceptible, though not marked, variation in the volume of urine in the several periods, the change being in the reverse direction from that observed in the first experiment. In other words, the volume of urine was larger with the smaller phosphorus supply. The fact that the low phosphorus ration in this experiment carried the larger amount of digestible protein may in part account for this increase in the volume of urine.

An examination of the figures shows that the volume of milk was somewhat larger during the low phosphorus period, and that there was a small but perceptible lowering, for a time, at least, of the percentage of fat in the milk. These changes are small, however, and not nearly as emphatic as was the case in the first experiment. (See Tables XXII–XXIV.)

6. *Effect of the two rations upon production.* — While the production of total milk solids, as well as of casein and fat, varied somewhat in the different periods, the data do not consistently show that these changes were due to the influence of the food. (See Table XXIV.)

Experiment 3 (1905). — This was a repetition of Experiment 1, and involved marked variations in the phytin content of the rations. In the experiment of 1904 (Experiment 1), in which the rations were chiefly made to differ by the substitution of washed for unwashed wheat bran, thus causing very large variations in the phytin content of the food, certain very remarkable results were obtained, which should not be accepted as final without substantiation through further observations. Consequently, it seemed wise to repeat the phytin experiment, and, as the animal used in Experiment 2 had become accustomed to experimental conditions and to the washed bran ration, it was decided to continue work with her.

The components of the ration, outside of the bran, were oat straw, rice, and wheat gluten. To these basal feeds washed bran was added in certain periods and unwashed bran in others. In the first experiment, when the change was made from the washed bran to the unwashed bran ration, hominy feed was substituted for the rice meal. In this experiment this substitution did not take place, but the rice meal was continued in both rations. By this arrangement the variations in the nucleo-proteid content of the rations were not emphasized by the substitution of hominy meal for the rice. As in the other experiments the protein supply was regulated by varying the quantity of wheat gluten.

Character and sequence of the rations. — Reference to the nucleo-proteid experiment shows that the cow during the last period of that experiment was fed the washed bran ration. It was necessary, therefore, that in the first period of this second phytin experiment there should be fed a ration containing unwashed bran.

Ration 1. — At noon, on February 16, the cow was suddenly changed to the ration containing the unwashed bran. She consumed the entire ration on the 16th, and two portions of it on the 17th, and refused the third portion. It was thought that possibly the quality of the bran was the cause of this refusal to eat, and, consequently, a bran of superior quality, procured from a nearby mill, was tried, but with no avail. The cow continued to refuse the ration during the 18th and 19th. On the 20th she was offered small portions of washed bran, which were eaten with a keen appetite. It was clearly indicated that the sudden introduction into the ration of certain bran constituents was responsible for the change in the animal's appetite. Her health did not seem to be impaired. She was not feverish, and, while her fæces had become somewhat softened in consistency, there was nothing in her outward appearance that indicated an abnormal condition of health. During this time of impaired nutrition the flow of milk was considerably lessened, as was the excretion of fæces and urine.

Ration 2. — Fed from noon, February 20, to the morning of February 25. The change back to the washed bran ration was sudden, the entire daily portion being wholly consumed. A normal milk flow was soon re-established.

Transition period. — This was begun at noon, February 25, and was completed on the morning of March 9. An attempt was made to transfer the animal from Ration 2 to Ration 1 by the daily substitution of two pounds of unwashed bran for an equivalent weight of washed bran, with an accompanying daily decrease of one-half pound of wheat gluten. This method appeared to be successful up to the point where the cow was receiving six pounds of unwashed bran. The first day that eight pounds

of unwashed bran was fed, the ration was not wholly consumed. The next day the cow again refused to eat, and it became necessary to place her back on a ration containing four pounds of unwashed bran and six pounds of the washed. This mixture was heartily eaten, and from this point the food was slowly changed to the high phytin ration by the daily addition of one pound of unwashed bran, and some days only one-half pound, with a corresponding decrease in the washed bran. In this way the transition was successfully accomplished.

Ration 1. — Fed from noon, March 9, to the morning of March 19.

Transition period. — From noon, March 19, to the morning of March 23. During this time two pounds of washed bran were substituted daily for two pounds of the unwashed, and when the ration contained six pounds of washed bran, one-half pound of wheat gluten was added daily to supply the deficiency in protein. At the end of this period the faeces had become hard and dry.

Ration 2. — From noon, March 23, to the morning of April 10.

Ration 1. — At noon, April 10, the cow was suddenly transferred to Ration 1, which she ate heartily for two days, and then refused further portions. She was then placed on a ration containing four pounds of unwashed bran and six pounds of washed bran, which she completely consumed on April 12.

Transition period. — From noon, April 12, to the morning of April 18. Starting with the ration which was fully consumed on April 12, a change was made gradually to Ration 1 by the daily substitution of one pound of unwashed bran for an equivalent quantity of the washed, the wheat gluten being decreased by one-half pound.

Ration 1. — From noon, April 18, to the morning of May 2.

Transition period. — This occupied from noon, May 2, to the morning of May 6. This transition was accomplished by the daily substitution of two pounds of washed bran for an equal weight of the unwashed, with a daily increase during the entire period of one-half pound of wheat gluten.

Ration 2. — From noon, May 6, to the morning of June 4. After June 4 the animal was placed with the Station herd, and slowly brought to the ordinary milk ration.

During this experiment a sudden change from the high phytin ration to the one containing washed bran was found to be inadvisable. Abundant evidence was accumulated concerning the drier condition and firmer consistency of the faeces when the washed bran was fed, apparently establishing beyond question the comparatively laxative effect of the whole wheat bran.

TABLE XXV.

THE RATIONS FED.

Ingredients.	Ration 1.	Ingredients.	Ration 2.
Oat straw	10 lbs.	Oat straw	10 lbs.
Bran	10 "	Washed bran . . .	10 "
Rice meal	7 "	Rice meal	7 "
Wheat gluten . .	1½ "	Wheat gluten . .	2 "
Total	28½ lbs.	Total	29 lbs.

TABLE XXVI.

THE COMPOSITION OF THE FEEDING STUFFS USED.

Feeds.	Water.	Protein.	Fat.	Phosphorus.		
				Total.	Soluble.	Inorganic.
	per cent	per cent	per cent	per cent	per cent	per cent
Oat straw	10.62	5.74	1.80	0.198	0.132	0.088
Bran	9.06	14.25	4.40	1.270	0.899	0.000
Washed bran . .	8.44	11.12	4.19	0.145	0.050	0.000
Rice meal . . .	12.67	8.37	0.36	0.084	0.007	0.000
Wheat gluten . .	7.41	85.50	1.56	0.220	0.032	0.000

TABLE XXVII.

QUANTITIES AND PERCENTAGES OF DIGESTIBLE NITROGEN AND DRY MATTER FED.

Date.	P fed.	Dry matter daily.			Nitrogen daily.		
		Fed.	In fæces.	Digested.	Fed.	In fæces.	Digested.
	grams	grams	grams	per cent	grams	grams	per cent
March 12–18 . .	77	11484	3523	69.3	284.7	83.9	70.5
March 30–April 5 .	16	11805	4282	63.7	269.0	82.6	69.3

TABLE XXVIII.

PERCENTAGE DAILY COMPOSITION OF THE MILK WITH BOTH HIGH AND LOW PHOSPHORUS INGESTION, INCLUDING TWO TRANSITION PERIODS.

Date.	P fed daily.	In milk.					Date.	P fed daily.	In milk.				
		Proteids.	Casein.	Fat.	Sugar.	Total solids.			Proteids.	Casein.	Fat.	Sugar.	Total solids.
	grams	p. c.	p. c.	p. c.	p. c.	p. c.		grams	p. c.	p. c.	p. c.	p. c.	p. c.
Mar. 16	77	3.27	2.69	3.40	5.58	12.25	May 2	65	3.21	2.63	3.80	5.36	12.37
17	77	3.03	2.60	3.25	5.78	12.06	3	54	3.11	2.57	3.65	5.46	12.22
18	77	2.93	2.33	3.25	5.42	11.60	4	43	3.09	2.54	3.80	5.37	12.26
19	65	2.92	2.37	3.20	5.34	11.46	5	31	3.16	2.66	3.55	5.35	12.06
20	54	2.91	2.38	3.30	5.35	11.56	6	16	3.26	2.71	3.65	5.30	12.21
21	43	3.03	2.48	3.40	5.29	11.72	7	16	3.24	2.63	3.45	5.38	12.07
22	31	3.00	2.47	3.20	5.44	11.64	8	16	3.34	2.75	3.00	5.33	11.67
23	20	3.06	2.47	3.15	5.46	11.67	9	16	3.32	2.68	3.75	5.20	12.27
24	20	2.94	2.44	2.95	5.61	11.50	10	16	3.18	2.51	3.35	5.23	11.76
25	18	3.04	2.48	3.25	5.43	11.72	11	16	3.29	2.70	3.20	5.32	11.81
26	18	2.96	2.39	3.15	5.44	11.55	12	16	3.30	2.72	3.15	5.30	11.75
27	18	2.96	2.41	3.20	5.40	11.56	13	16	3.29	2.72	3.10	5.39	11.78
28	18	3.03	2.44	3.15	5.37	11.55	14	16	3.24	2.65	3.25	5.51	12.00
29	18	3.01	2.44	3.20	5.40	11.61	15	16	3.22	2.63	3.10	5.29	11.61
30	16	3.03	2.47	3.20	5.47	11.70	16	16	3.16	2.60	3.30	5.67	12.13
Apr. 30	77	3.11	2.58	3.80	5.27	12.18	17	16	3.15	2.63	3.10	5.58	11.83
May 1	77	3.26	2.75	3.75	5.43	13.44							

TABLE XXIX.

PERCENTAGES OF VARIOUS FORMS OF PHOSPHORUS IN THE MILK.

Date.	P fed daily.	In milk.			Date.	P fed daily.	In milk.		
		Total.	Soluble.	Inorganic.			Total.	Soluble.	Inorganic.
	grams	per cent	per cent	per cent		grams	per cent	per cent	per cent
Mar. 16	77	0.101	0.076	0.071	Mar. 24	20	0.104	0.083	0.077
17	77	0.101	0.077	0.074	25	18	0.104	0.083	0.074
18	77	0.101	0.078	0.069	26	18	0.109	0.082	0.077
19	65	0.106	0.084	0.076	27	18	0.109	0.085	0.078
20	54	0.107	0.083	0.076	28	18	0.107	0.083	0.079
21	43	0.105	0.085	0.078	29	18	0.105	0.084	0.076
22	31	0.107	0.085	0.075	30	16	0.104	0.082	0.081
23	20	0.110	0.085	0.077					

TABLE XXX.

TOTAL PHOSPHORUS BALANCE.

Date.	Daily income and outgo of phosphorus.				
	Fed.	Total outgo.	In fæces.	In milk.	In urine.
	grams	grams	grams	grams	grams
March 12–18	77	68.4	43.5	14.7	10.17
March 30–April 5 . .	16	26.8	10.9	15.8	0.08

TABLE XXXI.

DISTRIBUTION AND BALANCES OF NUCLEO-PHOSPHORUS.

Date.	Total P fed daily.	Daily income and outgo fixed phosphorus.				
		Fed.	Total outgo.	In fæces.	In milk.	In urine.
	grams	grams	grams	grams	grams	grams
March 12–18 . . .	77	23.2	13.4	9.9	3.5	0.00
March 30–April 5 .	16	9.6	9.4	6.1	3.3	0.00

TABLE XXXII.

DISTRIBUTION AND BALANCES OF SOLUBLE ORGANIC PHOSPHORUS.

Date.	Total P fed daily.	Daily income and outgo soluble organic phosphorus.				
		Fed.	Total outgo.	In fæces.	In milk.	In urine.
	grams	grams	grams	grams	grams	grams
March 12–18 . . .	77	50.2	1.58	0.53	1.05	0.00
March 30–April 5 .	16	2.6	1.86	0.69	1.17	0.00

TABLE XXXIII.

DISTRIBUTION AND BALANCES OF INORGANIC PHOSPHORUS.

Date.	Total P fed daily.	Daily income and outgo inorganic phosphorus.				
		Fed.	Total outgo.	In fæces.	In milk.	In urine.
	grams	grams	grams	grams	grams	grams
March 12–18 . . .	77	4.0	53.4	33.1	10.2	10.10
March 30–April 5 .	16	4.2	15.5	4.2	11.2	0.08

TABLE XXXIV.

RELATION BETWEEN NITROGEN AND PHOSPHORUS EXCRETION.

Date.	Nitrogen.		Daily income and outgo of phosphorus.					
	Fed daily.	Outgo daily.	Fed.	Soluble fed.	Inorganic fed.	Total excreted.	Soluble[1] excreted.	Inorganic excreted.
	grams	grams	grams	grams	grams	grams	grams	grams
March 12–18 . .	284	241	77	54.0	4.0	68.0	45.0	43.0
March 30–April 5	269	246	16	6.8	4.0	26.8	17.3	15.4

[1] The soluble includes the inorganic.

TABLE XXXV.

EFFECTS OF THE INGESTION OF PHOSPHORUS COMPOUNDS UPON THE COMPOSITION OF THE MILK.

Date.	Total P fed daily.	In milk.				
		Proteids.	Casein.	Fat.	Sugar.	Solids.
	grams	grams	grams	grams	grams	grams
March 12–18 . .	77	3.07	2.53	3.28	5.56	11.91
March 30–April 5 .	16	3.05	2.45	3.09	5.46	11.59
April 24–May 1 .	77	3.22	2.66	3.73	5.47	12.41
May 29–June 4 . .	16	3.27	2.75	3.29	5.50	12.07

TABLE XXXVI.

EFFECTS OF THE INGESTION OF PHOSPHORUS COMPOUNDS UPON THE YIELD OF MILK AND MILK SOLIDS AND UPON THE EXCRETION OF URINE.

Date.	P fed daily.	Yield milk daily.	Daily yield milk solids.			Weight urine daily.
			Casein.	Fat.	Total solids.	
	grams	grams	grams	grams	grams	grams
March 12–18 . .	77	14606	58.0	478	1739	9433
March 30–April 5	16	14899	57.6	459	1726	5482
April 24–May 1 .	77	12686	53.0	473	1575	....
May 29–June 4 .	16	11917	51.7	392	1438	....

Discussion of results. — The results secured in this experiment are in the main confirmatory of those obtained in Experiment 1.

1. *The comparative nutritive value of the rations.* — Both rations supplied an abundance of digestible nutrients. The dry matter digested daily from Ration 1 was 17.5 pounds and from Ration 2, 16.5 pounds, the amounts of digestible protein being 2.8 pounds and 2.6 pounds respectively. The difference between these two rations is not sufficient to in any way account for the physiological and other effects observed. (See Table XXVII.)

2. *The amounts and forms of ingested phosphorus in the two rations.* — Ration 1 carried 77 gm. of phosphorus and Ration 2, 16 gm. Of

the phosphorus in Ration 1, 65 per cent or 50.2 gm. consisted of soluble organic phosphorus compounds, which was practically all phytin. In Ration 2 only 2.6 gm. of phosphorus was found in soluble organic combinations. The nucleo-proteid phosphorus in Ration 1 amounted to 23.2 gm. or 30 per cent of the whole, and in Ration 2, 9.6 gm. or 60 per cent of the whole, the larger amount of this form of phosphorus in Ration 1 being due to the difference in the composition of the unwashed and washed bran. As in former experiments, the food supply of inorganic phosphorus was small and similar in the two rations. These figures make it evident that the quantities and proportions of phosphorus compounds in these two rations were quite similar to what was found in the rations used in Experiment 1. (See Tables XXXI–XXXIII.)

3. *The relation in amounts and forms of the ingested and the outgoing phosphorus.* — The comments offered under this heading in discussing Experiment 1 could be repeated here in the main as accurately characterizing the outcome of this experiment. Briefly stated, the amount of outgoing phosphorus rose and fell with the food supply; during the feeding of the high phytin ration phosphorus storage occurred, while with the low phytin ration the outgo of phosphorus was an average of 10.8 gm. per day more than the income, a condition which the animal sustained through one period of thirty days without serious effects; the phytin phosphorus and that in the unused nucleo-proteids were practically all reduced to inorganic forms; the variations in the outgoing phosphorus were chiefly changes in the proportions of the inorganic salts of this element and finally no evidence was secured showing that there was any synthesis of the phosphorus-bearing proteids. (See Tables XXX–XXXIII.)

4. *Distribution of outgoing phosphorus compounds in the milk and egesta.* — Again the outcome of this experiment is similar to what was observed with the first one, viz., the rise and fall of phosphorus compounds in the egesta occurred chiefly in the fæces, though to a considerable extent in the urine. When the larger amount of phosphorus compounds was fed, there was a marked increase in the inorganic phosphorus compounds of the fæces and urine. Casein secretion was not affected by the phosphorus supply and the proportions of organic phosphorus bodies in the egesta appeared not to be affected by the supply of these in the food. (See Tables XXVIII and XXXI–XXXIII.)

5. *Physiological effects due to variations in the phosphorus bodies of the two rations.*— A larger proportion of Ration 1 was digested than of Ration 2, due undoubtedly to the fact that the washed bran was less digestible than the unwashed. Practically the same proportion of nitrogen was digested from the two rations, but there was a somewhat greater storage of nitrogen compounds from Ration 1 than from Ration 2, a result evidently independent of the phosphorus supply. The withdrawal of the phytin from the ration again had the effect of causing marked constipation, although in this experiment, because of the precautions that were taken in arranging the transition periods, it was not found necessary to administer a purgative at any time. The laxative influence of the phytin seems to be well established, for it does not appear probable that in washing the bran other compounds of a laxative character were taken out.

The volume of urine was greatly decreased when the washed bran was substituted for the unwashed, the quantity with the high phytin ration being 72 per cent larger than when the phytin was withdrawn. This result is fully as marked in this experiment as it was in the former one.

It is very clear that in this experiment, as in the first one, the excretion of nitrogen bears no relation to the phosphorus outgo, a fact to which reference will be made later in our discussion.

The average weights of milk produced as given for the several periods do not show any influence arising from changes in the amounts of phosphorus compounds fed. When, however, we come to consider the records of milk flow immediately succeeding the change to the washed bran ration, the effect of the change is made very evident. Table XXXVII shows the milk yield through a transition period beginning March 16th and ending March 24th.

The figures show an unmistakable influence of the change in the phytin supply.

The influence of a change in the rations upon the composition of the milk, that is, upon the percentage of fat, is not as marked as in the first experiment. In Table XXVIII, where the daily composition of the milk is detailed, covering a period from March 16th to March 30th, during which time the ingested phosphorus was diminished from 77 gm. daily to .16 gm., there is shown a small but unmistakable temporary lowering of the percentage of fat. In the period from April 30th to May 17th, when a similar reduction was made in the ingested phosphorus, the effect upon the fat percent-

age is much more marked, amounting to from 0.5 to 0.7 per cent. During the lowering of the quantity of phosphorus fed the decrease in the fat content of the milk was a progressive one. In this period there did not appear to be a tendency, as was observed in Experiment 1, towards the restoration of the fat content to its original

TABLE XXXVII.

YIELDS OF MILK DURING A TRANSITION PERIOD FROM HIGH TO LOW PHOSPHORUS RATION.

Date.	Total P fed.	Daily yield milk.	Date.	Total P fed.	Daily yield milk.
	grams	grams		grams	grams
March 15	77	14429	March 20	43.0	16202
16	77	14088	21	31.0	15167
17	77	14500	22	19.8	15181
18	65	15252	23	19.8	15365
19	54	15521	24	19.0	15408

proportions. The decrease in the percentage of milk solids is almost entirely accounted for by the decrease in the proportion of fat. (See Tables XXVII, XXX–XXXVI.)

6. *Influence of the two rations upon production.* — During this experiment the yield of total milk solids and of casein gradually diminished without any apparent relation between production and the character of the rations. The yield of milk fat was perceptibly lessened during the low phytin periods. (See Table XXXVI).

7. *Œstrum period.* — This animal did not show the marked disturbance in this direction that was observed with cow 1. On the twentieth of February, during the time she was passing from a low phytin ration to the one rich in that compound, the œstrum failed to appear. This was the only instance of the kind recorded with this animal. It was thought that perhaps a withdrawal of phytin from the ration for a long time would produce a result similar to that which occurred with cow 1, but during a thirty-day period of low phosphorus feeding, and afterwards, the œstrum regularly occurred. The animal afterwards became pregnant.

General Discussion of the Three Experiments.

The data obtained from these three experiments, involving the use of two animals, agree in consistently supporting the following conclusions. Certain of the facts observed, which, with others, are here briefly summarized, agree with observations made by other investigators:

1. The amount of outgoing phosphorus rose and fell with the quantity supplied in the food, though within narrower limits. When the phosphorus supply was abundant there was a storage of this element in the bodies of the animals, but during prolonged periods in which the supply of phosphorus was deficient there was withdrawn from the body store about 10 gm. daily in several periods.

2. Through catabolic changes the phosphorus of the phytin and that of the unused digested nucleo bodies was reduced to inorganic combinations, and was excreted chiefly in the fæces, though to a small extent in the urine. (The phosphorus of the urine is calculated wholly as inorganic. While we may not be justified in doing this, attempts to separate phytin or other organic forms of phosphorus from the urine by the methods employed in all the other work failed entirely. Whatever error is introduced by this method of calculation must certainly be small.) Further evidence of catabolic metabolism of phosphorus compounds is found in the fact that the inorganic phosphates of the milk were from three to five times greater in quantity than the total amount of such compounds in the food. The rise and fall in the amounts of outgoing phosphorus compounds occurred almost wholly with the inorganic salts found in the egesta. The organic phosphorus bodies of the egesta were but little affected, if at all, by the proportions of phosphorus compounds in the food. Variations in the phosphorus supply appeared not to modify the appropriation of this element by the milk.

3. No relation whatever appears to exist between nitrogen excretion and phosphorus excretion.

4. It is shown without question that the physiological effect of the two rations, due to the withdrawal from the bran of such compounds as were soluble in slightly acidulated water, differed to a marked degree. With the washed bran ration as compared with the one containing the unwashed bran, the following differences were observed:

a. Drier and much firmer fæces with the washed bran ration, accompanied by a constipated condition, requiring in some cases the use of a purgative.

b. A marked disturbance of appetite in Experiment 3, when a sudden change was made from the washed bran ration to the one containing the unwashed bran, indicating some specific physiological influence of the compound or compounds removed from the bran by leaching.

c. A greatly reduced flow of urine following a change from the unwashed bran to the washed bran ration, the reverse taking place when a reverse change was made.

d. An increase in the flow of mik consequent upon the withdrawal from the ration of the phytin and other water-soluble constituents of bran.

e. A reduction, sometimes large, in the percentage of fat in the milk consequent upon the withdrawal from the ration of phytin and other water-soluble constituents of bran.

f. A decreased production of butter fat during the period the washed bran ration was fed, notwithstanding a somewhat increased flow of milk.

g. The entire cessation of the œstrum period with cow 1 and a temporary disturbance of this period with cow 2.

h. The foregoing effects were observed chiefly in Experiments 1 and 3, in which the difference in the phosphorus content of the two rations was brought about by leaching the phytin and other soluble compounds out of the wheat bran. In Experiment number 2 where the phytin content was small and remained unchanged, similar physiological influences were not sufficiently marked to place much emphasis upon them.

To what cause shall these physiological influences be attributed?—We now come to the important question as to what compound or compounds in the rations may be regarded as the exciting cause of the physiological influences observed. It is doubtful whether our data justify definite conclusions, but the situation may be somewhat elucidated perhaps by a discussion of certain factors entering into the problem. There are several possibilities to be considered:

1. The difference in effect of the two rations may be due to the larger supply of phosphorus in one ration without reference to the form of combination.

2. The compound known as phytin may have specific physiological influences in several directions.

3. The withdrawal from the ration of the basic compounds with which phosphorus is associated in the compound phytin may explain wholly or in part the peculiar physiological influences observed.

In discussing the first point it should be stated that Experiment 2 was planned with the hope that by eliminating the phytin and varying the quantity of other phosphorus compounds light would be thrown upon the general question of the influence of the supply of organic phosphorus bodies without reference to their nature. The experiment was not entirely satisfactory. In no period was the phosphorus income equal to the outgo and it is not clear what would have been the result had the two rations been as fully differentiated in their phosphorus content as was the case in Experiments 1 and 3. However, there are indications, not to be ignored, that the two rations in the nucleo-proteid experiment had a somewhat unlike effect upon the flow and composition of the milk.

The other two points must be considered together. The problem here involved is whether if phytin exerts the physiological effects observed, its influence is to be attributed to its acid phosphorus radical or to the basic portion, calcium, magnesium, and potassium, or to the organic compound as an entity. The question was raised in the progress of the work whether the withdrawal from the bran by leaching of the bases calcium, magnesium, and potassium, might not so influence the supply of the compounds of these elements in the rations as to cause results that otherwise would be attributed to the phosphorus supply.

Analyses of both the unwashed and washed bran show that the leaching removed the greater portion of the magnesium and potassium compounds and but very little of the calcium compounds. The following figures confirm this statement:

TABLE XXXVIII.

INORGANIC CONSTITUENTS OF BRAN BEFORE AND AFTER LEACHING.

	P	CaO	MgO	K_2O
	per cent	per cent	per cent	per cent
Whole bran	1.42	0.182	0.894	1.58
Washed bran	0.145	0.380	0.162	0.084

The marked difference in the composition of the two kinds of bran, as indicated above, very naturally suggested the inquiry as to whether the supply of certain bases in the washed bran ration might not be insufficient. Consequently the amounts of the ingested and outgoing calcium, magnesium, and potassium oxides were determined for two periods in Experiment 3, one March 14 to 17, inclusive, during which time unwashed bran was fed; and March 30 to April 2, inclusive, this being part of a washed bran period. The results of this inquiry are given in the following tables:

TABLE XXXIX.

CaO Balance. Income and Outgo of Certain Bases.

Date.	P fed daily.	Daily income and outgo CaO.					Date.	P fed daily.	Daily income and outgo CaO.				
		Fed.	Total outgo.	In faeces.	In milk.	In urine.			Fed.	Total outgo.	In faeces.	In milk.	In urine.
	gm.	gm.	gm.	gm.	gm.	gm.		gm.	gm.	gm.	gm.	gm.	gm.
Mar. 14	77	31.7	36.8	11.0	25.2	0.6	Mar. 30	16	39.4	59.9	23.3	27.1	9.5
15	77	31.7	38.6	13.0	25.0	0.6	31	16	39.4	56.0	25.0	26.3	4.7
16	77	31.7	37.7	12.1	25.1	0.5	Apr. 1	16	39.4	60.0	21.9	27.3	10.8
17	77	31.7	36.7	12.4	23.9	0.4	2	16	39.4	58.0	25.8	26.0	6.4

TABLE XL.

MgO Balance.

Date.	P fed daily.	Daily income and outgo MgO.					Date.	P fed daily.	Daily income and outgo MgO.				
		Fed.	Total outgo.	In faeces.	In milk.	In urine.			Fed.	Total outgo.	In faeces.	In milk.	In urine.
	gm.	gm.	gm.	gm.	gm.	gm.		gm.	gm.	gm.	gm.	gm.	gm.
Mar. 14	77	68.3	45.1	36.0	3.6	5.5	Mar. 30	16	24.2	18.6	11.7	3.5	3.4
15	77	68.3	51.3	42.6	3.2	5.5	31	16	24.2	17.9	12.7	3.4	1.8
16	77	68.3	49.7	40.7	3.1	5.9	Apr. 1	16	24.2	14.2	7.0	3.5	3.7
17	77	68.3	46.8	39.8	3.2	3.8	2	16	24.2	16.6	11.7	2.9	2.0

TABLE XLI.

K_2O BALANCE.

Date.	P fed daily.	Daily income and outgo K_2O.					Date.	P fed daily.	Daily income and outgo K_2O.				
		Fed.	Total outgo.	In faeces.	In milk.	In urine.			Fed.	Total outgo.	In faeces.	In milk.	In urine.
	gm.	gm.	gm.	gm.	gm.	gm.		gm.	gm.	gm.	gm.	gm.	gm.
Mar. 14	77	166.7	140.7	49.8	28.1	62.8	Mar. 30	16	113.5	108.2	32.4	31.2	44.6
15	77	166.7	162.0	64.6	26.1	71.3	31	16	113.5	85.4	35.1	29.7	20.6
16	77	166.7	163.5	60.3	27.8	75.4	Apr. 1	16	113.5	81.8	22.9	32.7	26.2
17	77	166.7	146.7	54.7	26.4	65.6	2	16	113.5	81.6	37.9	31.2	12.5

The facts displayed in the above figures are very suggestive. It appears that in both periods the animal excreted more calcium oxide than she received, and the somewhat surprising result is shown that the animal gave up from her body store much the larger amount of calcium compounds when the income of phosphorus was less than the outgo. This fact will be considered later.

The supply of magnesium and potassium compounds was greater in the food in both periods than was the amount excreted. In fact there was a storage of magnesium in both periods and especially of potassium in the period of low phosphorus feeding. It is not easy to draw from these facts the conclusion that the unlike physiological influence of the two rations is due to a withdrawal from the bran of the bases under consideration. In both periods there was a calcium balance against the animal, and an apparently sufficient supply of both magnesium and potassium. Our knowledge of materia medica suggests, of course, that the larger supply of magnesium compounds in Ration 1 may have had some influence of a laxative character, and it is also conceivable that the greater amount of excreted potassium compounds may have influenced the flow of urine. Outside of these suggestions it is difficult to even theorize as to why the extent of the supply of these bases should explain the physiological influences that are under discussion. The authors are inclined to the view that the compound known as phytin exerts specific physiological influences.

The increased flow of urine, caused by feeding the unwashed bran ration. — Several points need consideration in this connection.

Marked changes in temperature undoubtedly have an influence on urine secretion, but as both of these animals stood in a room which was warmed to the point of comfort by artificial means, this factor hardly needs consideration. It has already been made clear that the larger urine excretion was not correlated with an increased nitrogen outgo.

The amount of water drunk would certainly exert some influence. In order to determine whether any relation existed between the water taken and urine excretion, the following table has been prepared, showing the average daily quantities of water consumed during the periods for which other averages have been given.

TABLE XLII.

RELATION OF FLOW OF URINE TO AMOUNT OF WATER DRUNK.

Cow 1.				Cow 2.			
Period.	P fed daily.	Water drunk daily.	Weight of urine daily.	Period.	P fed daily.	Water drunk daily.	Weight of urine daily.
	grams	lbs. oz.	grams		grams	lbs. oz.	grams
Mar. 11–16 . .	12.8	75 9	4974	Jan. 13–19 . .	18.2	88 ..	5752
April 12–18 . .	78.7	69 10	10555	Jan. 27–Feb. 3 .	37.3	78 5	4528
April 28–May 1	16.0	55 6	9560	Feb. 10–16 . .	20.2	85 12	5468
May 9–15 . .	83.3	97 12	12723	Mar. 12–18 . .	77.0	85 8	9433
May 22–26 . .	21.4	72 10	10147	Mar. 30–April 5	16.0	79 9	5482
Dec. 27–Jan. 2 .	37.3	77 15	3600				

It would not be logical to conclude from the figures of the above table that the variations in the volume of urine are caused by variations in the water supply. If the whole case rested on a comparison between the period May 9 to 15 and the periods preceding and following, a consistent relation would be established, but on the other hand a comparison of the period March 11 to 16 with April 12 to 18, and of February 10 to 16 with March 12 to 18, gives us opposing evidence.

The effects produced upon the volume and composition of the milk. — A definite explanation of the unlike influences exerted by the different rations upon the volume and composition of milk is not now possible. It may be that the volume relations of both urine and milk

are affected by the osmotic tensions induced by the presence of certain salts.

The work of Herter,[1] showing that an insufficient fat supply in the food of young animals depresses the assimilation of phosphoric acid is suggestive in this connection. These experiments show a reverse relation in that the elaboration of butter fat is apparently lessened by an insufficient phosphorus supply.

The question naturally presented itself as to whether the low phosphorus supply affected in any way the constitution of the milk fat. In order to obtain evidence on this point the butter fat was examined from five different periods in Experiment 3.

1. Fat from the milk of April 19 to 20. High phosphorus feeding immediately after the change from a low phosphorus ration.

2. Fat from milk of April 29 to 30, ten days after the change from a low phosphorus to a high phosphorus ration.

3. Fat from milk of May 7 to 8. Low phosphorus ration immediately after a change from the high phosphorus ration.

4. Fat from the milk of May 22 to 23, fifteen days after the change from a high phosphorus ration.

5. Fat from milk six weeks after the cow had been placed on the regular ration of the Station herd. This represents butter fat produced under entirely normal conditions.

The results of these examinations are given in the following table:

TABLE XLIII.

COMPOSITION OF BUTTER FATS.

No.	P fed.	Volatile acids.	Soluble acids.	Insoluble acids.	Iodine absorption.	Koet. No.	Melting point.
		per cent	per cent	per cent	per cent		
1	High	31.62	5.61	86.89	26.91	239	31.25
2	High	33.82	5.65	85.99	26.47	240	30.70
3	Low	29.60	4.91	87.40	25.41	236	32.44
4	Low	26.29	4.75	87.31	26.23	234	33.15
5	Normal	26.21	5.10	88.59	37.93	232	31.50

The indications are that the constitution of the milk fat was somewhat modified by the change in the rations. The volatile and soluble

[1] HERTER: Journal of experimental medicine, 1898, iii, p. 293.

fatty acids are in smaller proportion in the fat produced by the low phosphorus ration while the melting point is somewhat higher, facts that are entirely consistent with each other, and are an additional indication of a specific influence upon milk-fat secretion of the compounds taken out of the bran by leaching with water.

The source of the phosphorus given up from the body store during the periods of low phosphorus feeding. — Since these two animals were able to sustain a loss of phosphorus compounds from the body store during considerable periods of time without any apparently serious consequences to the general health, it becomes an interesting question as to what part of the body store was drawn upon. The fact that the nitrogen excretion was not increased during the feeding of the low phosphorus rations would indicate that there was no cleavage of the nucleo-proteid bodies stored in the tissues unless, indeed, it is possible for such cleavage to occur and leave the proteid component of these bodies intact.

It would appear reasonable to expect that the deficiency in the phosphorus compounds of the food would be made good from the body supply of what may be called the circulatory phosphorus compounds, that is, those which are soluble or transferable. Facts are available which enable us to consider this point of view more or less intelligently. Emmett and Grindley,[1] in their researches on the chemistry of the flesh of steers, have made a study of its phosphorus content in various forms. Their results show that the total phosphorus from such flesh soluble in cold water ranged from 0.146 per cent to 0.257 per cent, averaging approximately 0.2 per cent. Of this 0.12 per cent consists of soluble inorganic phosphorus bodies, chiefly potassium phosphates, only about 0.08 per cent being in the form of organic phosphorus compounds.

In an experiment by Jordan[2] concerning the relation of food to the growth and composition of the bodies of steers, it was found that the soft tissues of the bodies of the experimental animals, exclusive of the blood and the contents of the stomach and intestines, represented about 51 per cent of the total body weights. Assuming the same relations for the bodies of the animals in these experiments and that the soft tissues contain 0.2 per cent of phosphorus in soluble or transferable combinations, we find by computation that the total

[1] EMMETT and GRINDLEY: Journal of the American chemical society, 1906, xxviii, p. 25.

[2] JORDAN: Maine Agricultural Experiment Station. Report, 1895, p. 36.

quantity of such phosphorus would be approximately 525 and 450 gm. for cows 1 and 2 respectively. The figures of the preceding tables show that there was one period at least in which the phosphorus deficiency approximated 10 gm. daily for thirty days. The question is, then, whether from the store of soluble phosphorus compounds an amount equal to 300 gm. of phosphorus could be withdrawn without serious results to the animal's health. That this could happen certainly seems improbable.

Attention has been called to Tables XXXIX–XLI, which show the income and outgo of the oxides of calcium, magnesium, and potassium during two periods of four days each, one with an excess and the other with a deficiency, of phosphorus compounds in the ration. The unlike results in the two periods appear to be significant in their relation to the supply of phosphorus compounds. During the period of low phosphorus supply the withdrawal of calcium compounds from the body store was greatly increased, with a somewhat corresponding increase in the storage of potassium oxide. There was a storage of magnesium oxide in both the high and low phosphorus periods, this being considerably greater in the former. These facts would indicate that there was a replacement of calcium oxide by another base, particularly in the period of low phosphorus feeding. On the other hand the greatly increased excretion of calcium compounds in the low phosphorus period suggests a cleavage of calcium compounds in some way connected with the deficiency of phosphorus in the food, the most natural inference being that the egested phosphorus not supplied by the food was obtained from a body compound containing calcium oxide as a base.

The metabolism and excretion of phosphorus and other mineral compounds of the food. — It has been shown that the phosphorus of the catabolized phytin and nucleo-proteids appears in inorganic combinations mostly in the fæces, though to some extent in the urine, and that the calcium oxide which is withdrawn from the body store is excreted in a quite similar manner. These are facts of general physiological interest and are confirmatory of the observations made by other investigators.

The question as to where the cleavage of the compounds under consideration takes place is an interesting one. In order to throw light on this problem we have studied the action of trypsin and pepsin on the free acid of phytin and its simple salts without securing evidence of the power of these enzyms to split these bodies into

inorganic forms. To be sure, the influence of other enzyms, such as erepsin and of bacterial ferments, has not been tested and so we have no direct proof that these latter might not effect a cleavage of the phosphorus bodies we are studying, but this hardly seems probable.

It is certain that phytin entirely disappeared from the intestinal tract and did not reappear even in small proportions in the milk or urine. By our methods of analysis it is possible to determine very small amounts of soluble organic phosphorus but elaborate examinations of large quantities of milk, urine, and fæces, during a time when the animal was receiving an excessive amount of phytin, failed to reveal its presence even in minute proportions. These facts, together with the failure to cause a cleavage of phytin through the action of enzyms, indicate that this body was absorbed, metabolized, and its phosphorus excreted, largely through the fæces, in inorganic forms.

The observations of other experimenters show clearly that the presence of any compound in the fæces is not proof that it has not been absorbed into the circulation. Bergman[1] demonstrated that when glycero-phosphoric acid is injected subcutaneously into a sheep the phosphoric acid is excreted in the dung. Mendel and Thacher[2] have also shown that mineral compounds which are absorbed from the intestinal tract may afterwards appear in the fæces. There is a growing evidence among physiologists that the alimentary canal is a channel for the excretion of something more than the substances which have failed of digestion and absorption, and consequently that the percentage of digestibility of certain compounds is not measured by the difference between the amounts of these compounds in the food and in the fæces. It seems to be practically established that the claim made by Rubner[3] that the loss through non-absorption of the mineral constituents of whole wheat flour as compared with patent flour is much greater in the case of the former, does not rest on a sound basis, an observation which applies equally well to all conclusions reached in a similar manner. A comparison of the mineral compounds of the constituents of the food with those in the fæcal discharge evidently cannot be depended upon as a means of ascertaining the availability and usefulness of the ingested mineral compounds.

[1] BERGMAN: Jahresbericht für agriculturalische Chemie, 1901, iii, p. 354.
[2] MENDEL and THACHER: This journal, 1904, xi, p. 5.
[3] RUBNER: Zeitschrift für Biologie, 1883, xix, p. 45.

The supposed laxative effect of whole wheat bread. — It is generally believed, how correctly is not definitely established, that whole wheat bread is a desirable food for persons of a constipated habit. This supposed influence is usually attributed to the effect of the coarser material upon the peristaltic action of the intestines. The outcome of this investigation very naturally suggested the thought that if whole wheat bread really possesses the laxative properties assigned to it, this may be due to the amount of phytin it carries rather than to its mechanical condition. With this point in view, analyses were made of the milling products from a particular lot of wheat, which was ground at a nearby mill. These analyses are given in the table below:

TABLE XLIV.

PHOSPHORUS CONTENT OF WHEAT AND ITS MILLING PRODUCTS.

	P		P
	per cent		per cent
Whole wheat	0.378	First middlings[1]	0.071
Bran	1.280	Second middlings[1]	0.079
Middlings (ships)	0.857	Third middlings[1]	0.091
Germ	0.765	Germ roll flour	0.074
Straight flour	0.089	Tailings, or last roll	0.135
First break flour	0.089	Tailings, reel	0.134
Second break flour	0.088	Bran duster flour	0.196
Third break flour	0.088	Low grade flour	0.166

[1] "Middlings" is millers' term for fine flours.

It is evident, as is well known, that the phosphorus compounds of the wheat kernel are found mainly in the outer coatings and germ from which are derived the bran and middlings. As the bran phosphorus is mostly contained in the compound phytin, it is self-evident that this substance exists in much larger proportion in the whole wheat bread than in fine flour. These statements are offered as suggesting a problem for further study.

A pharmacological study of the compound phytin. — In view of the possible pharmacological properties of this compound, as indicated in these experiments, it was thought to be very desirable that experi-

enced investigators should undertake a study of its physiological influence. Correspondence was entered into with Dr. R. H. Chittenden of Yale University, and he very kindly consented to undertake pharmacological observations with this substance. There was prepared, therefore, in our laboratory the anhydro-oxy methylen di-phosphoric acid, which is phytin freed from its bases, a quantity of which was sent to Dr. Chittenden. We understand that the studies indicated are now in progress under the direction of Dr. L. B. Mendel.

The foregoing data include many interesting facts, but do not justify final conclusions concerning the main points under investigation. On the basis already established, however, the authors plan to continue the inquiry with the hope of reaching a more complete solution of the problems involved.

THE PHYSIOLOGICAL ACTION OF LANTHANUM, PRÆSEODYMIUM AND NEODYMIUM.[1]

By B. J. DRYFUSS and C. G. L. WOLF.

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THE salts which were here employed were used in preparations for atomic weight investigations, and while not of that degree of purity, were 98.0–99.0 per cent pure as far as their respective metals were concerned. They came to us in the form of the double nitrates of ammonium and the metal. The chlorids were prepared from these.

Experimental.

The standard solutions which were prepared for this work were made as nearly as possible isotonic with 0.6 per cent sodium chlorid solution. This was done empirically by preparing strong solutions of the salts of known concentration, and diluting till the freezing point corresponded to that of frog's blood. We found that solutions prepared on the calculation of the dissociation of a simple trivalent salt did not give, as might be expected, a solution corresponding to normal saline. In most instances these solutions were diluted with 0.6 per cent sodium chlorid. It is safe to assume that any effects here recorded are not hyper- or hypoisotonic effects.

All the water used was redistilled in glass, which is perhaps unnecessary for this type of work. The solutions of the chlorids of lanthanum, præseodymium and neodymium are acid, and have a markedly astringent taste.

[1] We are indebted to the American Welsbach Company through the kind offices of Professor Charles Baskerville, of the College of the City of New York, for the supplies of the rare earths which we have used. We have also to thank Professor Baskerville for the history of the preparations which we have studied.

Action on Proteins.

Serum from rabbit's blood was used. Solutions containing 0.8 per cent of lanthanum, præseodymium, and neodymium chlorids were added drop by drop. A thick white coagulum was formed in each case, which fell rapidly to the bottom of the tube. At a concentration of 0.01 per cent all the solutions produced decided coagula.

A 10 per cent solution of egg albumin was prepared. The solutions produced the same effect as with serum albumin. On dilution of the albumin solution, no difference in the power of precipitation of the different salts was noted. The addition of potassium citrate caused the precipitate to disappear immediately (12).

The albumoses of Witte's peptone were purified by precipitation with ammonium sulphate and dialysis. Traces of coagulable proteins were removed previously by acetic acid and heat. The albumoses were precipitated by alcohol and dried. A 1.0 per cent solution was prepared. The solutions of the chlorids all failed to produce precipitates with this solution.

A 1.0 per cent solution of Kühne's peptones was not precipitated by any of the salts.

Action on Unicellular Organisms.

Bacteria. — A suspension of bacillus subtilis was used, and equivalent solutions of the metallic chlorids containing 0.8 per cent of the metal were employed for the tests.

A loopful of the bacilli was placed in a solution of lanthanum chlorid, and allowed to remain there for thirty minutes. Microscopic examination showed few dead bacilli, many motile ones, and numerous spores. Some of the material which had been exposed to the solution was transferred to an agar tube. After twenty-four hours incubation there was a marked growth. Some of this culture was placed in lanthanum chlorid for ninety minutes. Microscopic examination showed no active organisms. No spores were seen. After this second treatment no growth could be obtained on agar.

With præseodymium chlorid more dead bacilli were found, and the growth on agar after the first exposure to the salt was less than that obtained with lanthanum.

Neodymium chlorid had a more destructive action than either of the preceding two salts. There was, however, a slight growth on agar after thirty minutes exposure to the solution.

Opalina. — On adding a drop of neodymium chlorid to a drop of solution in which were suspended some opalina ranarum, the movements of these organisms became jerky and spiral in character. The rate of movement was quickly reduced. This was followed by gyratory movements. These ceased after fifteen minutes. After the application of the salt solution vacuoles soon appeared, increasing rapidly in number. The cell wall assumed a lobular shape. The edges were crenated. The cytoplasm shrank from the cell wall.

Paramœcium. — When a solution of neodymium chlorid was added to paramœcium caudatum, the effect seemed to be more marked. Action commenced at an earlier stage than with opalina. The organism was, in the end, more resistant. These infusoria instantly became quiet. The only movement was that of the cilia. This was so slow that the action of each cilium could be observed. All pulsation of the vacuoles ceased at once. The ciliary movement at the periphery ceased in eighteen minutes, that at the peristome in twenty-two minutes. The cytostome retracted from contact with the trichocyst. Numerous vacuoles were present, and the nucleus became very prominent.

Vorticella. — This organism seems most susceptible to the action of these solutions, being killed by neodymium chlorid solution in eight minutes. The bell becomes round. The spiral pedicle contracts at once on application of the solution, but later extends. The alternate contractions and expansions are not as full as in the normal. There is some shrinkage of the endosarc. The peristome is puckered. In præseodymium solution, of the same strength, the organism lasted twelve minutes. In lanthanum chlorid, there was a faint response to stimulation after thirty-five minutes contact. In forty-five minutes the infusorium was dead.

As will be seen, the effects of equivalent solutions of præseodymium chlorid are like those of neodymium, but not so powerful. A paramœcium lasted thirty-three minutes as against twenty-two minutes with neodymium chlorid.

A solution of lanthanum chlorid did not paralyze the organism at once, as did the other two solutions. The infusorium darted to and fro for about sixty seconds, when it became quiet. It then underwent the same changes as when subjected to neodymium and præseodymium. In addition, a granular appearance opposite the peristome was noted.

In the case of the higher unicellular organisms, one may conclude

that the primary effect is one of paralysis of the excretory function, of which the lessened pulsation of the vacuoles bears evidence, as also do the granules found in the vacuoles.

The sudden stoppage of general movement, the gradual shrinkage of the cytoplasm, the loss of shape of the cell wall, and its crenation, show the effect of a powerful astringent. It would appear that this paralysis, which so rapidly ensues, is dependent on the astringent or coagulative property. This is also shown by the peripheral flagella which come in immediate contact with the solution. The motion of these is stopped, while those in the protected peristome continue in motion three times as long.

That cessation of vacuolar pulsation should take place so quickly, is to be explained on the theory that the solution passing through the peristome, and entering the delicate digestive tract, quickly paralyzes and destroys this apparatus, while the more dense cell wall is only affected later, and does not constringe as much or as quickly as the cytoplasm.

Blood cells. — A few drops of neodymium chlorid solution were added to some frogs' blood cells. After five minutes the cells became vacuolated. Poikilocytosis manifested itself. After fifteen minutes, the nuclei became very prominent. The walls of the cells burst, the contents escaping.

Effect on Muscle and Nerve.

The studies on muscle and nerve were directed towards observing, besides the comparative effect, the individual action on the muscle fibre, the nerve endings, and the nerve proper.

On muscle. — Two sciatic-gastrocnemius preparations were made. One was placed in isotonic neodymium chlorid solution, the other in isotonic sodium chlorid. In both cases the nerve was carefully isolated from the solution. The muscles were weighed, and the minimal intensity of current necessary to provoke contraction was measured. The results are shown in Table I.

The muscle which was immersed in the sodium chlorid was fresh and glistening at the end of eighteen hours, while that in the neodymium solution was white and opaque. Commencing opacity was observed two hours after immersion. The sudden fall in the indirect stimulation from 44 to 5, while the fall in the direct was

gradual, indicates a degenerative change taking place in the muscle itself.

A similar experiment with præseodymium chlorid gave the result shown in Table II.

With lanthanum the results shown in Table III were obtained.

The effect of the solutions on the type of the muscle curve was obtained by immersing a gastrocnemius in the solution and suspending the nerve outside the solution on a pair of gold-plated electrodes.

TABLE I.

Sodium Chlorid.				Neodymium Chlorid.			
Time.	Direct.	Indirect.	Weight.	Time.	Direct.	Indirect.	Weight.
3.10	27.5	44	2.92	3.06	20.0	44	2.46
3.25	27.5	44	....	3.20	19.0	44	....
3.40	26.2	44	....	3.39	18.0	44	....
3.50	23.0	44	....	3.45	13.0	44	....
4.07	23.0	44	....	4.20	12.0	44	...
4.30	22.0	44	....	4.35	10.5	44	....
5.00	22.0	43	3.09	5.01	6.0	5	2.36
22.50	21.0	43	....	22.50	0	0	....

The muscle was loaded with 10 gm. and afterloaded. After obtaining normal curves, the saline solution was replaced by the solution to be tested, without disturbing the muscle. An effect was soon noted. The latent period was increased and the length of the contraction diminished.

No period of increased excitability was obtained. The base line gradually rose, owing to the permanent contraction of the muscle. Using equimolecular solutions, it was found that the toxicity of the solutions increased with increasing molecular weight.

With neodymium solutions, and indirect stimulation every four minutes, it was found that the shocks were effective for a period of twenty to eighty minutes. The permanent contraction was also most marked in this solution. Muscles in præseodymium solution under similar conditions lasted one hundred and sixty-five minutes.

With lanthanum, the stimuli were effective for two hundred and twenty-five minutes.

In order to compare the effect on a poisoned limb, two methods were used. In the one case, the usual method was employed of ligaturing one common iliac, and injecting the poison into the dorsal

TABLE II.

Sodium Chlorid.				Praeseodymium Chlorid.			
Time.	Direct.	Indirect.	Weight.	Time.	Direct.	Indirect.	Weight.
11.05	70	88	1.36	11.05	70	88	1.26
11.20	70	88	....	11.20	68	88	....
11.40	70	88	....	11.40	65	88	....
12.00	70	88	....	12.00	50	85	....
12.30	70	88	....	12.30	50	85	....
13.00	70	88	....	13.00	40	85	....
13.20	70	88	....	13.20	25	85	....
13.25	70	88	....	13.25	15	85	....
13.50	70	88	....	13.40	55	80	....
14.30	60	88	....	14.10	0	2	....
14.45	60	88	....	14.45	0	0	....
14.48	60	88	....	....	....	....	....
15.35	60	88	1.52	....	....	....	1.25

lymph sac. At the end of thirty minutes, the two gastrocnemii were prepared, and introduced into a crank myograph. Tracings were made, maximal break shocks only being used. A Brodie cut-out key was employed, which was run by a motor. The difference in the reaction between the poisoned and unpoisoned limb was very marked. (See Table IV.)

The second method was that of Harvey Cushing. Removing the urostyle, the abdominal aorta was reached from the back. A cannula was introduced into the vessel, and the abdominal veins were cut. One of the limbs was protected by ligature of the common iliac artery of that side. The solution, colored with methylene blue, was then

allowed to perfuse through the unprotected limb, and the difference in the behavior of the two limbs noted. (See Table V.)

In all these experiments shocks were sent in every two seconds. In the latter series, responses were obtained at normal distance in the protected leg after the unprotected limb had been completely poisoned.

TABLE III.

Sodium Chlorid.				Lanthanum Chlorid.			
Time.	Direct.	Indirect.	Weight.	Time.	Direct.	Indirect.	Weight.
11.05	70	88	1.36	11.05	70	88	1.50
11.20	70	88	....	11.20	68	88	....
11.40	70	88	....	11.40	65	85	....
12.00	70	88	....	12.00	60	85	....
12.25	70	88	....	12.30	55	85	....
13.00	70	88	....	13.00	50	85	....
13.20	70	88	....	13.15	50	85	....
13.50	70	88	....	13.50	40	85	....
14.10	70	88	....	14.10	40	85	....
14.30	60	88	....	14.27	30	80	....
14.45	65	88	....	14.42	20	80	....
14.48	60	88	....	14.48	25	80	....
15.35	60	88	1.52	15.35	0	0	1.85

Nerve tissue. — A gastrocnemius-sciatic preparation was made, and placed in the moist chamber of a crank myograph. The nerve for about a length of 5 mm. was immersed in a shallow depression containing the solution. A stretch of nerve on both sides was armed with electrodes, and needle electrodes were also placed in the muscle tissue itself. By means of a key, any part of the preparation could be stimulated. A cut-out key was used, and the preparation received stimuli every two seconds. The distal end of the nerve rapidly lost the power of conveying stimuli to the muscle.

The following are the results with equimolecular solutions, and secondary coils at equal distances: Neodymium chlorid blocked the

impulses in four minutes. Præseodymium chlorid required eleven minutes. Lanthanum chlorid was effective in seventeen minutes.

Heart muscle. — The effect on heart muscle was examined by means of a heart lever, and also by the suspension method. The heart was

TABLE IV.

	Unprotected limb.			Protected limb.		
	Start.	Dead.	Interval.	Start.	Dead.	Interval.
$NdCl_3$	3.03	3.10	7′	3.19	3.40	21′
$PrCl_3$	12.08	12.22	14′	12.27	1.00	33′
$LaCl_3$	4.03	4.30	27′	4.35	5.06	31′

left in situ. The solution was dropped on the organ. The effect was much more rapid than with ordinary striped muscle. A reduction in the amplitude of the curve took place within five to ten seconds after applying the solution. The reduction in height was sometimes

TABLE V.

	Perfusion started.	Dead.	Interval.
$NdCl_3$	11.34	11.39	5′
$PrCl_3$.	11.40	11.57	17′
$LaCl_3$	11.08	11.31	23′

accompained by a slowing in rate from thirty-three to twenty-five beats per minute. At other times, the normal rate persisted almost to the end of the experiment.

What was quite noticeable was that minor curves intervened, giving dicrotic beats both in systole and in diastole. These corresponded to the peculiar contraction of the ventricle which is called peristaltic(13). The contraction at the apex was followed by another before the first had completely finished. This phenomenon has also been observed by Harnack in his work on the effect of copper salts on heart muscle. The auricles were much less easily affected than were the ventricles. Their pulsation continued long after the ventricles had ceased to

beat. Washing with sodium chlorid or with Ringer's solution was ineffectual in restoring the heart function.

With heart muscle, the difference in the effect of the solutions was as follows. Neodymium chlorid. Heart ceases beating in thirty-two minutes. Præseodymium chlorid. Heart ceases beating in forty-nine minutes. Lanthanum chlorid. Heart ceases beating in fifty-eight minutes. Normal heart, cessation in two hundred and ninety minutes. In the normal heart, the ventricles beat as long as the auricles. No peristaltic effect was noted.

General Effects.

In the effort to determine the general effect of these substances, they were administered mostly subcutaneously, in a few instances, intraperitoneally. The animals used were frogs, pigeons, rats, and guinea-pigs.

Finding that there was no result from the injection of a single dose of 0.020 gm. of the chlorids per kilo in rats, pigeons, and guinea-pigs, we carried out a series of experiments, in which daily doses of the above amount were administered.

A rat, weighing 120 gm., was given 0.0024 gm. of neodymium chlorid daily for forty-three days. At the end of this time it weighed 110 gm. It was in every way in the same condition as a control animal. The total amount administered was 0.103 gm. or 0.86 gm. per kilo.

A pigeon, weighing 310 gm., was given subcutaneously 0.0068 gm. daily for thirty days. At the end of this time it weighed 310 gm. Another pigeon, weighing 340 gm., received 0.0068 gm. of lanthanum chlorid daily for a month. Its final weight was 320 gm. The same lack of action was observed with lanthanum chlorid with rats and guinea-pigs. On section, the only effect, which could be traced to the solution was an area of induration at the seat of injection.

Effect of large doses. — A guinea-pig weighing 440 gm. received intraperitoneally 0.1 gm. of lanthanum chlorid at 2.49 P.M. At 3.10 the animal was in slight convulsions. At 3.20 the convulsions had increased in intensity. At 10 the next morning it was found dead. The autopsy showed the peritoneum to be slightly cloudy, and congested at the seat of injection. There was about 5 c.c. of fluid in the cavity. The heart was normal. There was a small hemorrhagic infarct in the upper lobe of the left lung, and also in the upper part of

the lower lobe of the right lung. There was slight congestion of the liver. The cortex of the kidneys was deeply injected. The pyramids were pale.

Summary.

Equimolecular solutions of the chlorids of lanthanum, præseodymium, and neodymium increase in toxicity with increasing molecular weight.

It is probable that part of the acute effect observed is due to the hydrolysis of these salts, and to the consequent toxicity of the acid produced.

It has not been possible to demonstrate a chronic condition of intoxication using moderate amounts of the salts.

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THE VOLUME CURVE OF THE VENTRICLES OF THE MAMMALIAN HEART, AND THE SIGNIFICANCE OF THIS CURVE IN RESPECT TO THE MECHANICS OF THE HEART-BEAT AND THE FILLING OF THE VENTRICLES.[1]

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Various Views as to the Filling of the Ventricles.

NEARLY three hundred years ago, Harvey described the filling of the ventricles of the heart as follows: "The blood enters the ventricles not by any attraction or dilation of the heart, but by being thrown into them by the pulses of the auricles; and when I

[1] A preliminary report on this subject was presented at the meeting of the American Physiological Society in December, 1904. This journal, 1905, xiii, p. 24.

speak of pulses as occurring in the auricles or ventricles, I mean contractions." Precisely the same idea as to the mechanical action involved in the filling of the ventricles is expressed in the best and most widely read of modern elementary text-books of physiology in these words: "At each systole of the auricles the ventricles are filled, and the auricles emptied."[1] Thus, down to the present day, a great number, perhaps a majority of all the students who acquire the elements of physiology are taught that the systole of the auricles fills the ventricles. Hill says that even at the present time, "little can be added to Harvey's admirable description."[2]

It is probable that Harvey based his description of the mechanics of the filling of the ventricles more upon such animals as the frog, than upon the mammalian heart. It is only with difficulty that the eye can follow the complicated movements of the mammalian heart. Indeed Harvey himself was at one time almost inclined to think with Frascatorius, "that the motion of the heart was only to be comprehended by God." In such an animal as the frog, on the contrary, the relations of the movements of the various parts of the heart are more easily followed.[3] Even an unskilled observer with a frog's heart beating before him can see, or believes that he sees (once the genius of Harvey has shown him what to see), that the contraction of the auricle fills the ventricle. Indeed it is probable that the persistence with which the idea that the systole of the auricles plays an important part in the filling of the ventricles so long held its place in the minds of physiologists, was due to their familiarity with the almost diagrammatic picture which the frog's heart seems to afford.[4]

[1] HUXLEY'S Lessons in Elementary Physiology, first published in 1866, repeatedly revised by HUXLEY, then by Sir MICHAEL FOSTER and Dr. SHERIDAN LEA, and finally by Dr. F. S. LEE, last American edition, 1905, p. 80.

[2] LEONARD HILL in SCHÄFER'S Text-book of physiology, 1900, ii, p. 2

[3] It must be remembered that when HARVEY denied that "the ventricles are filled by any attraction or dilatation" of their own, he was addressing the disciples of GALEN, who believed in a suction action of the heart very different from that which some modern authorities deduce from GOLTZ and GAULE'S discovery of a "negative pressure" in the ventricles.

[4] An extraordinary example of the persistence of this view is afforded by LANDOIS'S Text-book of Human Physiology. This work has been through ten large editions in the original German, and numerous translations. Yet it teaches (American edition, 1904, p. 97) that "the effect of the auricular contraction is the distension of the relaxed ventricles which in small measure are dilated by the elastic traction of the lungs." Accompanying this statement in a manner to

Modern investigators have almost without exception recognized that auricular systole is only an accessory factor in the mechanics of the mammalian heart. The importance assigned this factor has almost invariably diminished with each successive writer. According to the description recently published by Howell[1] the filling of the ventricles is accomplished thus: "As soon as the ventricular relaxation is complete the pressure of blood in the auricles opens the auriculo-ventricular valves, and from that moment until the beginning of the auricular systole the blood from the large veins is filling both ventricles and auricles. The ventricles become more tense and the auriculo-ventricular valves are floated into position ready for closure.[2] The auricular systole sends a sudden wave of blood into the ventricles, dilating them still further." Again Howell speaks of auricular systole as "a rapid peristalsis which sweeps a portion of the blood before it into the ventricle." In respect to the increased distention of the ventricles produced by auricular systole it may be added that Erlanger,[3] in whose experiments the movements of the heart were recorded by means of tambours, found — and his curves demonstrate — that "it sufficed to record the movements of the right ventricle alone when the auricles were beating vigorously, for under such circumstances the contractions of the auricles send distinct waves into the ventricles." Since a wave does not necessarily involve a flow, the question offers itself, — Does this wave which auricular systole causes to sweep over the ventricles and which is so striking a feature in the appearance of the heart's activity, really produce any considerable increase in the volume of the ventricles? The evidence presented in this paper shows that it does not.

In a review of the literature of the Diastole of the Heart by Ebstein[4] which has recently appeared in the Ergebnisse der Physiologie, one hundred and eighty-seven papers are quoted. They present a bewildering array of experiments, observations, and theories,

remove all question as to its meaning are two diagrammatic representations of the mammalian heart, which exhibit the filling of the ventricles precisely as this event appears in the frog.

[1] Howell, W. H.: Text-book of physiology, 1905, pp. 480 and 488.

[2] Such a movement of the auriculo-ventricular valves was demonstrated by the present writer by means of a working model of the heart before the American Physiological Society in December, 1904. *Loc. cit.*

[3] Erlanger, J.: This journal, 1906, xvi, p. 163.

[4] Ebstein, E.: Erbegnisse der Physiologie, Dritter Jahrgang, ii Abteilung, pp. 121–194.

They seem to justify the position of Tigerstedt[1] who, after mentioning the physical factors which may be supposed to take part in the movement of the blood from the auricles into the ventricles, has expressed the conclusion that in the present state of our knowledge the relative importance of these factors cannot be determined with any precision. No extended critique of the literature will here be attempted. Only a few of the more distinct theories will be quoted. These are selected with reference to the distinctness of the picture which they afford of the flow into the ventricles, rather than for an explanation of the force or forces which cause the flow. Diagrams are also herewith presented showing the form which, as it seems to the writer, the cardiac plethysmogram would have according to the various theories.

In Fig. 1 is expressed the manner of the filling of the ventricles according to the statement of Harvey already quoted. The initial down stroke represents the systolic emptying of the ventricles. The succeeding horizontal line shows the collapsed and empty condition of the chambers. The sudden rise, occupying only a tenth of a second, is the refilling of the ventricles by auricular systole.

Brücke[2] in his celebrated theory of the "Selbststeuerung des Herzens," held that the ventricles are distended by the pressure of the blood transmitted through the coronary vessels from the aorta. Under these conditions the refilling would begin immediately after the completion of systole. The rate at which the heart would be distended would depend largely upon the rate at which the muscle fibres relax and yield to the stretching force. Brücke's explanation of the part played by auricular systole is not accessible to the writer. Fig. 2 seems with this omission a fair interpretation of Brücke's theory.

Roy and Adami[3] describe the manner in which they believe the ventricles to be filled, (Fig. 3) as first a rapid inflow in the early part of diastole, then a slower flow or cessation of flow during the latter part of diastole, and finally "a phase of increased rapidity of flow . . . owing to the successive contraction of the veins and auricles." Such a description certainly leaves much to be desired. It is not quantitative. What fraction of the entire volume of the ventricles runs into

[1] TIGERSTEDT, R.: Lehrbuch der Physiologie des Menschen, 1897, i, p. 157.

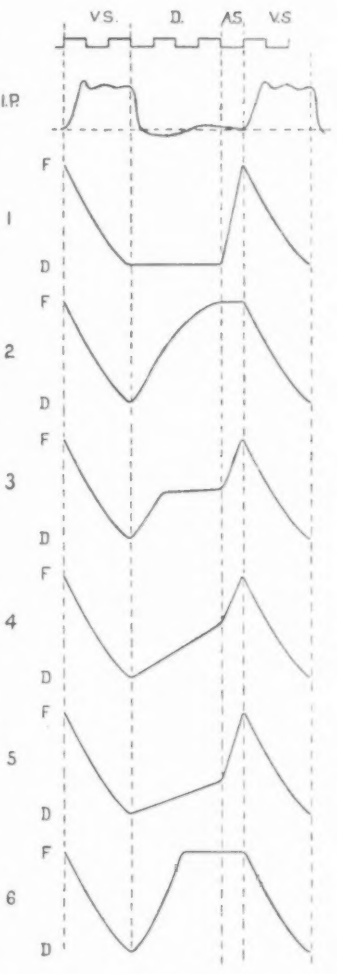
[2] For statements of BRÜCKE'S views see TIGERSTEDT'S Lehrbuch des Kreislaufes, 1893, pp. 51 and 52.

[3] ROY and ADAMI: Practitioner, 1889, xliv, p. 170.

the relaxed chambers during the early part of diastole? What is the volume of blood which the auricles force into the ventricles to complete their distention? Is it 20, 40, 60 or 80 per cent of the volume which the ventricles discharge in their systole?

Porter[1] says that an instant after the ventricles have completed systole and have begun to relax, "the auriculo-ventricular valves must open and permit the auricular stream to resume its flow. From this point until after the next ventricular systole has begun . . . blood flows into the ventricles." Hürthle[2] has given a similar description of the period of the filling of the ventricles. (Fig. 4.)

François-Franck[3] recognizes two periods in the filling of the ventricles. "A first gradual inflow commences at the moment of ventricular relaxation and lasts until the instant of auricular systole; the repletion is then completed by the tide of blood sent into the ventricle by the auricle at the instant of auricular systole." François-Franck accompanies these statements with the diagram herewith reproduced (Fig. 7). This diagram, if it be taken as representing the volume changes of the ventricles alone, seems to the present writer to



FIGURES 1–6.—The periods of the cardiac cycle, intraventricular pressure curve (I. P.), and six cardiac plethysmograms, showing the forms which (as it seems to the writer) this curve would assume according to the statements of (1) Harvey, (2) Brücke, (3) Roy and Adami, (4) Porter and Hurthle, (5) François-Franck, (6) Luciani. (F, filled; D, discharged.)

[1] PORTER, W. T.: Journal of physiology, 1892, xiii, p. 535.

[2] HÜRTHLE: Archiv für die gesammte Physiologie, 1891, xlix, p. 84.

[3] FRANCOIS-FRANCK: Travaux du laboratoire de Marey, 1877, iii, p. 321. See also Archives de physiologie, 1890, pp. 395–410.

be wholly irreconcilable with the description above quoted. While the description would correspond to Fig. 5, the diagram is essentially similar to Fig. 2. Such a curve indicates (1) that at the moment of ventricular relaxation, the blood rushes into the ventricle in such volume that the ventricle is more than two-thirds filled in the first third of diastole; (2) that in the next third of diastole, the filling of the ventricle is completed by a gradually lessening stream through the auriculo-ventricular valves; (3) that the filling of the ventricle is therefore complete before the occurrence of auricular systole. Since the volume curve runs parallel to the abscissa at the period of auricular systole, it signifies that the auricular systole injects no appreciable quantity of blood into the ventricle.

Luciani[1] believes in an active diastolic expanding force in the myocardium. He describes the cardiac plethysmogram as (1) a steeply descending line corresponding to the systolic discharge, (2) a steeply ascending line (often exhibiting notches) corresponding to the active diastole, and (3) a gradually ascending line (sometimes horizontal or even slightly descending) corresponding to the passive diastole and pre-systole, or contraction of the auricles. As this is a description of the plethysmogram of the whole heart, Luciani points out that at the instant of auricular systole the curve does not truly show the movement of blood between the auricle and ventricle. (Fig. 6.)

The cardio-pneumatic curves have been regarded by most writers as expressing the variations in the volume of the blood in the thorax. Haycraft and Edie,[2] however, regard them as oncometric records of the cardiac volume. In many cases these curves reveal an up stroke at the very beginning of diastole as steep and as high as is the down stroke corresponding to the systolic discharge.

Finally, mention must be made of the conclusions reached by Otto Frank[3] which, although based on experiments on the excised frog's heart, are highly significant for the dynamics of the mammalian heart as well. They have been summarized by Burdon Sanderson[4] as follows: "(1) The isometric curve of muscle is represented by the pressure curve of the systole of the charged ventricle with arrested

[1] Quoted from EBSTEIN'S review in the Ergebnisse der Physiologie, 1904, iii, 2, p. 181.

[2] HAYCRAFT and EDIE: Journal of physiology, 1891, xii, p. 426.

[3] FRANK, OTTO: Zeitschrift für Biologie, 1895, xxxii, p. 370.

[4] SCHAFER'S Text-book of physiology, 1900, ii, p. 442.

discharge; (2) the isotonic curve of muscle is represented by the volume curve of the ventricle; the load, by the resistance in front as measured by the aortic pressure; (3) as in muscle the isometric curve culminates before the isotonic, so in the ventricle the isometric pressure curve culminates sooner than the isotonic volume curve. The pressure curves and volume curves recorded in a ventricle working under the conditions which exist in the circulation, resemble in general the after-loading curves of muscle. . . ."

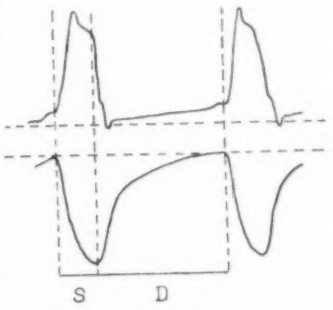


FIGURE 7. — Reproduction (reduced one-third) of a diagram by François-Franck in the Travaux du Laboratoire de M. Marey (1877, III, p. 321), entitled "Schéma de l'opposition des courbes des pulsations du cœur (ligne supérieure), et des courbes des changements de volume (ligne inférieure); *D*, periode de réplétion diastolique; *S*, periode d'évacuation systolique."

PREVIOUS PLETHYSMOGRAPHIC INVESTIGATIONS.

The method employed by the majority of the investigators, who in modern times have studied the mechanics of the heart, has consisted in recording the pressure changes in the chambers of the heart and in the blood vessels. Undoubtedly it is true, as Porter expresses it, that "the filling of the heart is the result of differences of pressure between the great veins, auricle, and ventricle." The measurement of these pressures has been accomplished with such perfection of technic as to leave little need of improvement. Yet the conclusions in respect to the filling of the ventricles to which such investigations have led, leave much to be desired. For the study of this problem the writer therefore had recourse to a method of recording the volume changes of the heart, — the pressure curves being recorded synchronously merely to show the periods.

The majority of previous volumetric observations upon the mammalian heart have been directed toward determining the gross change in volume (the volume of the systolic discharge) rather than to the details of the form of the volume curve, — as for instance, in the investigations of Johansson and Tigerstedt.[1] The tracings obtained

[1] JOHANSSON and TIGERSTEDT: Skandinavisches Archiv fur Physiologie, 1889, i, p. 331; and 1891, ii, p. 409.

by Roy and Adami[1] by means of their cardiometer show little detail of contour. Indeed it would seem that the large and rapid volume changes of the heart could scarcely be transmitted with any fineness of detail by a medium of transmission which (for this purpose at least) is as heavy and viscid as the oil with which their cardiometer was filled. In our experiments the medium of transmission was air. It may be added, however, that many of the tracings obtained by Roy and Adami by means of their myocardiograph (showing the cyclic changes in the various axes of the heart) seem to the writer to be in accord with the data presented in this paper.[2]

A considerable number of plethysmograms have been published by Stefani.[3] Upon them Luciani based the generalizations already quoted. These volume curves were obtained from dogs by means of a fistula in the pericardial sack. Through the fistula the pericardium was distended with air. The volume changes of the heart were recorded by means of a tambour connected with the fistula. While some of Stefani's tracings are essentially similar to those given in the present paper, others exhibit a wide variety of forms.

As early as 1877 François-Franck[4] had obtained graphic records of the volume changes of the dog's heart and of the ventricles of the human heart from a case of ectopia cordis. Upon these data he based the diagram and description already given. In some of his experiments on dogs the pericardial sack was used as the cardiometer. In others a glass vessel was slipped over the heart and made air tight by tying the pericardium over the free edge of the vessel. This cardiometer was then connected with a tambour to which the volume changes of the heart were transmitted by the air.[5]

The methods employed by Stefani and by François-Franck (except in the case of ectopia cordis) involved the enclosure of the entire

[1] Roy and Adami: Philosophical Transactions, 1892, clxxxiii, B, p. 202.

[2] This similarity is especially evident in the records in which the heart was slowed by stimulation of the vagus. Roy and Adami: *Loc. cit.*, p. 223.

[3] Stefani: Archives italiennes de biologie, 1893, xviii, p. 119; and 1889, xxxii, p. 439.

[4] François-Franck: *Loc. cit.*

[5] Hill and Barnard employed a tennis ball, tying the pericardium over the ball to render the window air tight. British medical journal, 1897. Dagobert Blank employed a cardiometer similar to that of François-Frank. Inaugural Dissertation, Gottingen, 1905. Knoll employed a method similar to that of Stefani. Sitzungsber. d. Kaiserl. Akad. d. Wiss. Wien, 1880, Math. naturw. Kl.

heart, auricles as well as ventricles, within the air space connected with the recording tambour. Under such conditions a movement of blood from the auricles into the ventricles would, theoretically at least, have no effect whatever upon the volume curve. Whether the auricles discharge a large volume of blood into the ventricles or none at all, the volume curve would in either case run parallel to the abscissa. These methods therefore can yield no conclusive evidence with regard to the movements of the blood through the auriculo-ventricular orifices during the period of auricular systole. The volume curves to be given in this paper were obtained by means of a plethysmograph or cardiometer in which the ventricles only were included.

Method of Experimentation.

All the experiments were performed upon dogs. The animals received a moderate dose of morphine, and were then anæsthetized with ether administered by means of a vaporizing flask connected with a tracheal cannula. In the first series of experiments upon twenty-five dogs artificial respiration was administered by means of a hand bellows. In the second series upon twenty dogs, advantage was taken of the immense improvement in technic originated by Sauerbruch [1] and simplified by Brauer.[2] Thus when the thoracic cavity was opened the tracheal cannula was immediately connected with a T-tube of which one opening led to a large gasometer (supplied by a pump) containing air at a constant pressure of 10 mm. of mercury. From the other opening of the T-tube, the expired air was allowed to escape through a Mueller valve properly adjusted. Under these conditions the lungs are distended to about their normal capacity and follow the respiratory movements of the ribs and diaphragm so well that the animal continues to breathe in a wholly natural manner even when, as in some of these experiments, the period of observation lasts for many hours.

The T-tube and tracheal cannula must not be directly connected. If they are, the volume of the stationary air is so reduced that the animal respirates itself into acapnia and thus into shock. If, however, the cannula and T-tube are connected by a wide tube, 25 to 50 cm. in length, shock does not occur.

[1] Sauerbruch: Mitteilungen aus den Grenzgebieten der Medizin und Chirurgie, 1904, xiii, Art. xvii.

[2] Brauer: *Ibid.*, 1904, xiii, Art. xviii.

The thorax was opened by an incision through all the costal cartilages at their junctions with the ribs and through the ensiform appendage. Hemorrhage was prevented by placing a ligature about the internal mammary arteries and veins at a point close to the origin of the former. The sternum and costal cartilages, with the adherent muscles and skin, formed a lid which could be lifted to allow the adjustment of the cardiometer upon the ventricles. After this adjustment the lid was closed down, the crack along its edge packed with cotton wool, and the animal covered with a cloth to avoid cooling.

The cardiometers employed were constructed from light rubber balls. It was important that they should neither be so large as to encroach seriously upon the space needed by the lungs, nor so small as not to give ample room for the ventricles to expand to the fullest extent. It was necessary, therefore, to have half a dozen of these balls ranging in diameter from 7 cm. to 11 cm. In each ball a round window (of a diameter 0.7 that of the ball) was cut. Over the window a piece of sheet rubber (dental-dam) was cemented. The centre of this curtain was then cut out, leaving a hole (of a diameter 0.6 that of the window). In the opposite side of the ball a glass tube was inserted which connected the interior of the ball with a rubber tube (10 mm. interior diameter and 120 cm. long), which led to the recording tambour. One or the other of two tambours (10 cm. and 12 cm. diameter respectively) was used according to the size of the animal. They were similar in construction to the Marey tambour of ordinary size. Each was capable of registering a volume considerably greater than was ever required in the observations, without stretching the rubber enough to involve any appreciable rise or fall of pressure. By means of a large syringe they were tested as to their capacity to follow large and rapid volume changes. The stylus of the tambours was found to follow, without appreciable lag, movements of the plunger of the syringe of considerably greater volume and rapidity than those occurring in the experiments. After each experiment the tambour was graduated by connecting it with a flask into which water was measured from a burette. Thus a scale in cubic centimetres was drawn on the smoked paper.

Before the adjustment of the cardiometers upon the heart, the pericardial sack was cut open, so that it could be rolled back entirely off the auricles as well as from the ventricles. All risk of compression of the auricles or veins was thus avoided. The cardiometer was

then slipped over the ventricles until the curtain around the window fitted snugly in the auriculo-ventricular groove. The circular curtain, while exerting only a very slight pressure, nevertheless fitted sufficiently well to prevent any leak of air in or out, as could be readily tested after death.

Validity of the Volumetric Method.

The question now arises, Can the volume changes of the exterior of the ventricle be taken as identical with the volume changes of the ventricular chambers? The adjustment of the cardiometer over the ventricle is shown in Fig. 8. From this figure it can be seen that any movement of the ventricle inward or outward through the window of the cardiometer would occasion a serious error in the volume record. The utmost care was taken, therefore, to adjust the cardiometer so that the rubber curtain should hold the window in an unvarying position upon the auriculo-ventricular groove.

Figure 8. — Diagrammatic representation of the construction of the cardiometers employed and of their adjustment upon the ventricle. The rubber stopper cut, inserted, and cemented around the glass outlet tube (as shown) made this joint tight. The arrangement of the rubber curtain over the window is described in the text.

In order that the exterior and interior volume changes of the ventricles shall be identical, it is essential that the septum formed by the closed mitral and tricuspid valves shall remain practically flat. Roy and Adami [1] believe that the papillary muscles draw the flaps of the valves inward, and thus assist in emptying the ventricles. If such is the case, the true rate of the systolic discharge will not be correctly portrayed in the volume curve of the exterior of the ventricles. During diastole, however, no error is thereby threatened.

Finally, it is obvious that changes in the volume of blood in the coronary vessels will influence both the form and amplitude of the volume curve.[2]

[1] Roy and Adami, Practitioner, 1890, xliv, pp. 88–94.

[2] Direct evidence on the flow in the coronary circulation is afforded by the graphic record of Chauveau and Rebatel, reproduced in Tigerstedt's Lehrbuch des Kreislaufes, 1893, p. 53.

Technic of the Pressure Curves.

Intraventricular pressure was recorded by means of a metal catheter passed into the left ventricle by way of the carotid.[1] In the first series of experiments a Gad metal membrane manometer was employed; in the second series, a Hürthle spring manometer. Magnesium sulphate solution was the medium of transmission.

Porter has shown that in records of the intraventricular pressure changes "the height of the [systolic] up stroke and the depth of the [diastolic] negative pressure cannot be correctly given in the same curve."[2] In respect to the record of diastolic pressures a systolic "up stroke of correct height is the first evidence of a false curve." Our interest lay primarily in the events of diastole. In respect to systole distinct indications of the points of onset and completion of the period were the only features of importance. In our experiments the manometers were for these reasons either left entirely undamped, or were damped as slightly as was compatible with the indication of these points. To this lack of damping is due the large inertia distortion in the up stroke of many of our pressure curves. Moreover, if the converse of Porter's statement be also true, the incorrect height of the up stroke in our curves indicates their approximate accuracy in respect to the pressures during ventricular diastole. On this view the absence of any negative pressure in some of our curves and the brevity of its duration in all others demonstrate that in these experiments the ventricles on passing into diastole exerted no suction sufficient to be considered as an important factor in the filling of their chambers.

Graphic Records of the First Series of Experiments.

An example of the tracings obtained in the first series of experiments is given in Fig. 9. The meaning of such a record is to be gathered by carefully noting the synchronous relations of the pressure and volume curves. Taken together these curves afford an expression of the mechanics of the heart similar to that which an engineer obtains in the indicator diagram of a steam engine.

[1] The catheter must be bent. Otherwise the displacement of the heart by the cardiometer may cause the catheter to puncture the posterior wall of the ventricle. If bent too much the catheter by pressing upon the interventricular septum is liable to induce a discharge of extra-systoles, as in Fig. 13.

[2] Porter, W. T : *Loc. cit.*, p. 521.

Period of discharge. — We may conveniently begin the interpretation of the records at the instant (marked 1 in both curves) before the occurrence of ventricular systole. The ventricles are full. The volume curve is at its highest point. With the onset of systole the pressure within the ventricle rises abruptly. But for an instant longer the volume is not diminished. Then as the pressure reaches a point (between 1 and 2) at which it exceeds the pressure in the arteries, the volume curve starts downward. The abruptness with which the descent begins indicates the suddenness with which the semilunar valves are thrown open, and the blood is shot into the arteries. The steepness of the descent shows the rapidity with which 90 per cent of the discharge is accomplished. This portion of the descent is almost a straight line; therefore the rate of discharge is uniform. As it nears the bottom of the descent, the volume curve rounds away to a blunt point (3 to 4). Thus the discharge slows, and then ceases. At the corresponding point (4) in the pressure curve, the pressure suddenly falls. This shoulder at the end of the pressure "plateau" is generally recognized as marking the completion of systole and the closure of the semilunar valves. That this shoulder and the blunt point of the volume curve occur together affords further evidence in favor of this view. The column of blood rushing through the semilunar orifices does not suck blood into the arteries after the contraction of the heart muscle is at an end, as was believed by Moens.[1] If such were the case the point would succeed the shoulder. The ventricle does not continue in systole after the discharge is ended, as held by Roy and Adami,[2] otherwise the point would precede the shoulder.

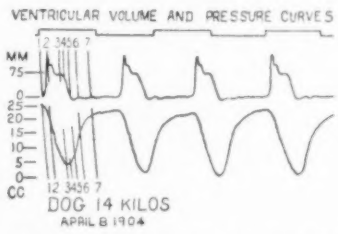


FIGURE 9. — About five-eighths the original size. Time record in 0.5 second. Pressure record from the left ventricle. The up stroke of this curve is distorted by an entire lack of damping (see page 336 of text), yet the negative pressure indicated is slight and its duration brief. The lower record is the volume curve of the ventricles. The zero level in the graduation of this and succeeding curves represents merely a convenient line of reference, having no determined relation to entire emptiness of the ventricular chambers.

[1] MOENS: Archiv für die gesammte Physiologie, 1897, xx, p. 531.

[2] ROY and ADAMI: Practitioner, 1890, xliv, p. 87.

A small dose of atropin, however, induces the latter condition by interfering with the diastolic relaxation of the ventricle. When this drug is administered the amplitude of the plethysmogram of the dog's heart is greatly diminished while the form of the curve is very similar to that shown in Fig. 1.

Period of filling. — It is probable that for a brief period immediately following the completion of systole, during which ventricular pressure falls to the abscissa, all the heart valves are closed (4 to 5). Yet comparison of the pressure and volume curves shows that during this period the latter rises sharply. This initial increase in the bulk of the ventricles is probably due to the rush of blood from the aorta into the coronary vessels which are now released from the compression incident to systole. In many records a slight notch in the volume curve marks the end of this brief period.

At the instant that ventricular pressure reaches the abscissa (at 5) a further rise in the volume curve occurs. At this instant therefore the auriculo-ventricular valves are opened by the pressure of the blood in the auricles, and the refilling of the ventricular chambers commences. From this point the volume curve swings upward, and by a line steep and often nearly straight almost to its summit, then curving quickly until parallel to the abscissa, it expresses the refilling of the ventricles. The rapidity of the inflow indicated by this rise of the volume curve is startling; for it shows that the refilling of the ventricles occurs early in diastole, and that it is as rapid a process as is the systolic emptying.

Period of rest. — During the latter part of diastole,[1] the volume curve runs parallel to the abscissa, indicating that during this period no movement of blood into the ventricle occurs (from 7 on). It is well recognized that this period is of very variable duration. Differences in the rate of the heart beat are effected almost entirely by lengthening or shortening this period. In a very rapidly beating heart it entirely disappears. The volume curve then becomes simply a series of sharp up and down strokes following each other in immediate and rapid succession.

Auricular systole. — If the contraction of the auricles drives blood before it into the ventricles, the volume curve must show, just prior to the down stroke of ventricular systole, a rise proportional to the

[1] The term "diastasis" might be employed to indicate this "period of rest," and to distinguish it from diastole, the "period of relaxation and refilling."

volume of blood which the auricles inject. In many volume records no such rise occurs. In others, as in the record under discussion, a slight wave in the volume curve coincident with auricular systole may be seen. It indicates, apparently, that the contraction of the auricles increases the ventricular volume to the extent of a few drops at most. Often the volume curve falls again after this auricular rise and before the onset of ventricular systole. There is, indeed, a chance for error in the volume curve during this period; but the probable error will be to exaggerate, rather than to obscure, the auricular rise. As the cardiometer tilts the ventricles upward, their weight rests in part upon the auricles. Thus the contraction of the latter may, and frequently does, lift the heart somewhat further into the cardiometer. When this error is avoided by care in the adjustment of the instruments, the graphic records show that the systole of the auricles moves at most only a fraction of a cubic centimetre of blood into the ventricles.

> It appears very probable that the wave which auricular systole induces and which presents so deceptive an appearance as it travels rapidly over the ventricles, must cause the complete closure of the auriculo-ventricular valves which were probably floated into position ready for closure by the cessation of the inflow at 7.[1]

This description of the volume curve holds true only when the heart is beating vigorously and under conditions as near the normal as possible. After extensive hemorrhage, for example, the amplitude of the heart beat is diminished, and the filling of the ventricles may occur in two distinct stages. The volume curve then takes a form similar to Fig. 3. It may be that this indicates that, when the pressure of the blood in the veins is insufficient to distend the ventricles to their full capacity, the "peristalsis-like wave of the auricular contraction" assists in the filling of the ventricular chambers.

Second Series of Experiments.

In Fig. 10 is represented an example of the records obtained in the second series of experiments. As already stated, the animals were maintained by a natural respiration of compressed air. Intraventricular pressure was recorded by a Hürthle manometer. In ad-

[1] See footnote number 2 on page 327 of this paper.

dition to the volume curve, obtained as in the earlier experiments, the movements of the auricle were also recorded. For this purpose a miniature cardiometer (2 to 3 cm. in diameter) was slipped over the right auricular appendage,[1] and connected with a small tambour. In the auricular records the down stroke after the diastolic pause marks the contraction of the auricular appendage; the succeeding up stroke its relaxation. The waves which follow in many of the tracings were caused by the movements of the ventricles, and are of no present interest. Comparison of the synchronous lines in Fig. 10 exhibits the same relations between the pressure and volume curves as those already noted in Fig. 9. The volume record, however, after the rapid inrush of blood in the early part of diastole, rises in this particular case more gradually than in most of the records obtained. This is probably due to a more gradual relaxation of the cardiac muscle, — an observation for which the variations in the rate of relaxation often observed in striated muscle furnishes an analogy. The onset of auricular systole occurred at the point marked 1. A scarcely perceptible wave in the volume curve developed at this point. The pressure within the ventricle was not perceptibly increased. The volume of blood which was moved through the auriculo-ventricular valves by the auricular systole was therefore practically nil.

FIGURE 10. — About five-eighths the original size. Time in 0.5 second. Pressure record from the left ventricle (manometer very slightly damped). Record of movements of right auricular appendage. The auricular curve is drawn upon a much larger scale than is the volume curve of the ventricles below it, to which alone the scale graduated in c.c. applies.

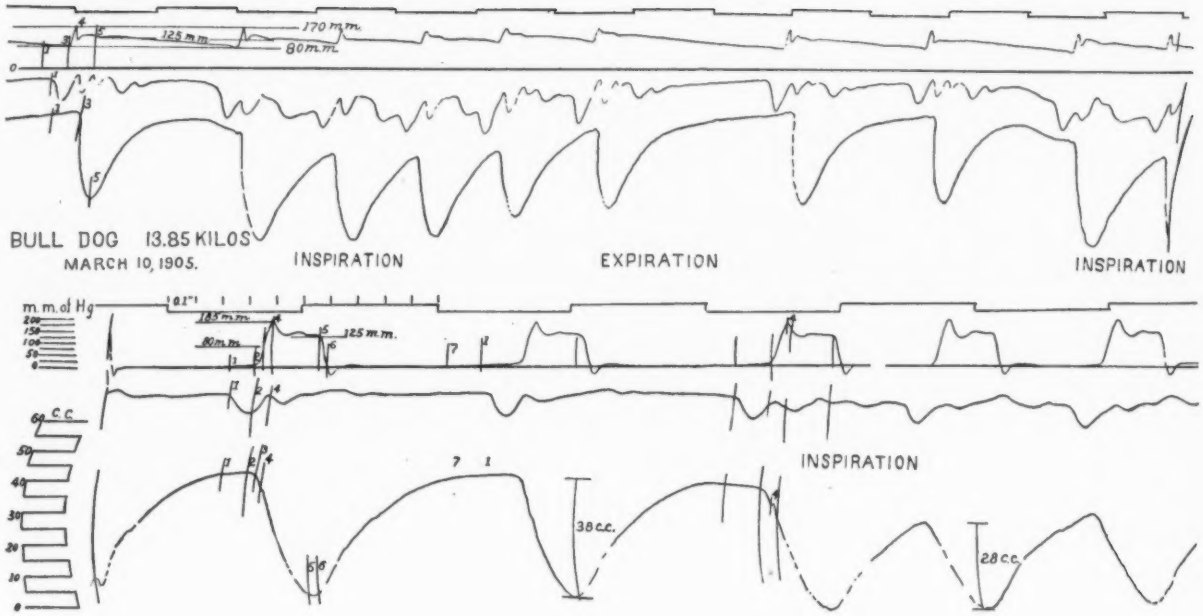
A more detailed study of the relations of pressure and volume is afforded by Figs. 11 and 12. In the upper record (Fig. 11) aortic pressure, the auricular movements and the ventricular volume are shown. Noteworthy are the differences in the volume of the systolic discharge of the rapidly succeeding heart-beats accompanying inspiration, and of the slow but full beats occurring between respirations.

[1] This cardiometer was held in place by a ligature placed upon the tip of the auricular appendage and drawn out through the small opening of the cardiometer before this opening was connected with the tambour.

In this record the volume curve is distorted by the movements of the diaphragm against which the cardiometer was pressed. Immediately after obtaining the record, the cardiometer was readjusted upon the heart so as not to be thus affected. The catheter connected with the manometer was passed through the aortic valves into the left ventricle. The kymograph was set for a somewhat higher speed; and the lower record (Fig. 12) was obtained.

Comparison of the three curves in each record and of the two records with each other shows that the commencement of auricular systole occurs at the point marked 1. At 2 ventricular systole begins; at 3 the pressure in the ventricle has risen to 80 mm., and equals the pressure in the aorta. The semilunar valves are opened at this point and aortic pressure rises rapidly. At the same instant the volume curve begins to descend, indicating the commencement of the ventricular discharge. At 4 a slight notch in the volume curve occurs; at the corresponding point ventricular pressure reaches the peak (185 mm.) which precedes the plateau; and arterial pressure attains the summit of the primary pulse wave (170 mm.). At 5 systole is complete. Ventricular pressure falls below aortic pressure (125 mm.). At this instant occur in striking coincidence the blunt point of the volume curve, the shoulder of the ventricular pressure curve, and the dicrotic notch in the aortic pressure curve. At 6 ventricular pressure has fallen to the abscissa, and as the ventricles rapidly refill the volume curve moves upward. At 7 the ventricles are full. Neglecting the refilling of the coronary vessels the volume curve indicates that 19 c.c. of blood has entered each ventricle. From this point onward until the onset of the next ventricular systole no increase in ventricular volume occurs. There is, however, a slight rise of ventricular pressure above the abscissa coincident with auricular systole. Together these two observations indicate that the ventricles are already filled so completely that auricular systole in this case does not inject even a fraction of a cubic centimetre of blood, although the force of its contraction is sufficient to cause a slight rise of ventricular pressure. It seems probable that under these conditions the auriculo-ventricular valves are closed at the completion of auricular systole, which occurs two or three hundredths of a second before the onset of the next ventricular systole.

After obtaining the record above discussed, the instruments were again adjusted. As a result of this readjustment the ventricles executed after each regular systole an extra-systole as shown in Fig. 13. Records


170 m.m.
125 m.m.
80 m.m.
BULL DOG 13.85 KILOS
MARCH 10, 1905.
INSPIRATION
EXPIRATION
INSPIRATION
m.m. of Hg
185 m.m.
80 m.m.
125 m.m.
c.c.
INSPIRATION
38 c.c.
28 c.c.

FIGURES 11 and 12. — About one-third the original size. Time in 0.5 second. The speed of the kymograph was considerably greater in Fig. 12 than in Fig. 11 (129 and 77 mm. per second respectively).

The manometer was damped to record systolic pressures as accurately as possible. In Fig. 11 aortic pressure was recorded, in Fig. 12 that of the left ventricle.

The graduation and numbering is the same in both records. Comparison of these two figures shows (in addition to their significance as explained in the text) the character of the errors to which the volumetric methods here employed are liable. The auricular curve (just below the pressure base line) in Fig. 11 is influenced by the ventricular as well as by the auricular movements. After adjustment it recorded (Fig. 12) the auricular movements alone. The ventricular volume record (the lowest curve) in Fig. 11 gives an incomplete measure of the volume changes as compared with the volume record in Fig. 12, because the cardiometer was not adjusted so that the rubber curtain fitted up near the auriculo-ventricular groove. Furthermore, this curve in Fig. 11 is distorted by the movements of the diaphragm, which push the cardiometer further up over the ventricles, and therefore raise the volume curve at each expiration. Such errors are readily recognized, and thus easily avoided.

In both figures the synchronous lines marked 1 indicate the onset of auricular systole. At 2 occurs the rise of ventricular pressure marking the onset of ventricular systole. At 3 the semilunar valves are opened and the discharge begins. The volume curve starts downward, the aortic pressure upward, while the ventricular pressure rises above 80 mm., and thus exceeds aortic pressure. At 4 occur the crest of the pulse wave, the ventricular pressure peak, and a notch in the volume curve. At 5 the dicrotic notch in the aortic curve, the shoulder at the end of the ventricular pressure plateau (both at a pressure of 125 mm.), and the blunt point of the volume curve. At this instant, therefore, occur the end of systole, the completion of the discharge, and the closure of the semilunar valves. At 6 ventricular pressure has fallen to zero, and the volume curve starts upward, indicating the opening of the auriculo-ventricular valves and refilling of the ventricles. At 7 the volume curve ceases to rise. The ventricles are so completely filled that the succeeding auricular systole raises ventricular pressure slightly, but does not increase the volume.

similar to the pressure curve here given have been published by Marey from a horse, and by Hürthle from a dog. In pathological conditions, such as mitral stenosis, extra-systoles are of not uncommon occurrence. The volume curve in the record shows that the extra-systole, following the regular systole before the ventricles have had time to fill, causes only an inconsiderable discharge into the arteries. The refilling of the ventricle after the extra-systole is accompanied by a negative wave in the auricular curve. The three beats here shown occurred in the pause between respirations. During inspiration the record (not shown) was entirely similar to the third, fourth, and fifth heart-beats in Fig. 12.

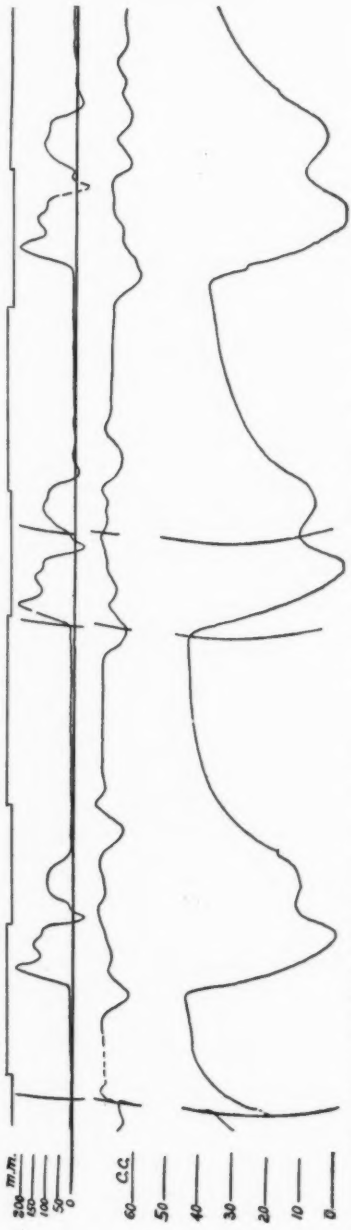


FIGURE 13. — About one-third the original size. Identical with Fig. 12, except that, owing to a readjustment of the catheter, the ventricle discharged a submaximal extra-systole after each regular systole during the respiratory pause. See footnote 1 on page 336.

RESPIRATORY VARIATIONS IN THE VOLUME CURVE.

The extent to which variations in the volume discharge of the ventricles may occur synchronously with the respiratory movements, is shown in Fig. 14. Examination of this record shows that the differences in the volume curve during the different phases of respiration are entirely dependent on the rate of the heart-beat. The amount of the discharge of each of the narrow beats occurring during inspiration is diminished not because the ventricles relax more slowly, or are filled less readily, but because one

beat follows the preceding before the ventricles have had time to relax. The picture of the respiratory differences in the heart-beat afforded by this record is probably somewhat exaggerated from the normal. But the heart-beats accompanying and immediately following inspiration are, except in a very slowly beating heart, always distinctly smaller than the slower beats between respirations. The up strokes of the volume curve expressing the filling of the ventricles are as distinctive and almost as uniform as are the systolic down strokes. The differences in the volume curves during the successive phases of the respiratory cycle are due entirely, therefore, to the variations in rhythm. The rapid sequence of the systoles accompanying and following inspiration causes the systolic down stroke of one beat to set in before the diastolic up stroke from the preceding beat is complete. The slow sequence during the respiratory pause, on the other hand, allows a sufficient interval between successive beats for the completion of the up stroke, or, in other words, for the relaxation of the muscle fibres of the walls of the ventricles and the filling of the ventricular chambers.

This record shows also that the inspiratory increase in rate of beat induces the phenomenon of Treppe in the musculature of the ventricles resulting in a more complete emptying of the ventricular chambers by each systole. Owing to a similar Treppe, or more probably, to an increase of tonus in the auricles, the cardiometer placed on the right append-

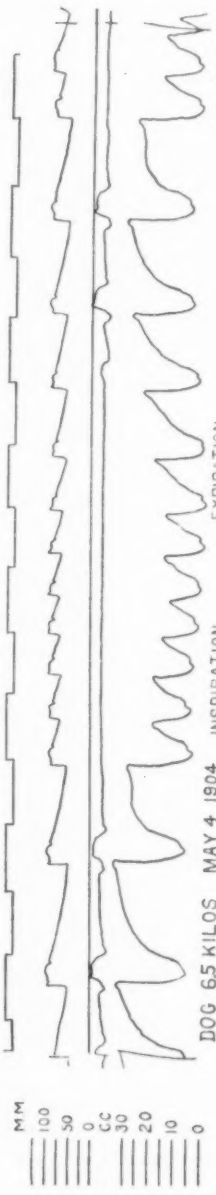


FIGURE 14. — About three-fifths the original size. Time record in 0.5 second. Aortic pressure curve. Movements of right auricular appendage. Owing to an increase of auricular tonus during the inspiratory increase in cardiac rhythm the auricular record is incomplete. The volume curve of the ventricles exhibits extreme respiratory variations.

age was not air tight during the inspiratory period. Hence the failure to record auricular systole during this period.

It is sometimes taught that the inspiratory rise of arterial pressure is due not only to the increased rapidity of beat accompanying inspiration, but also to a greater amplitude of the inspiratory beats induced by the increased negative pressure in the thorax.[1] No such increase in amplitude occurs under the conditions of these experiments. Indeed it cannot occur even when the thorax is intact; for the extent to which the ventricles fill is determined wholly by the length of time allowed for the relaxation of their walls to develop. Cardiac muscle, like striated muscle, at the completion of a contraction does not pass instantly into a condition of flaccidity; but relaxes at a rate determined by intracellular events. There must indeed be some extending force, corresponding to the weight which is hung on the ordinary muscle preparation in isotonic contraction curves. Within wide limits, however, the relaxation of a muscle is not perceptibly influenced by variations in the extending force. The rate and extent of the filling of the ventricles is correspondingly independent of variations in the venous pressure (which is their distending force), so long at least as the supply of blood flowing from the veins into the auricles is not diminished, nor its pressure considerably lowered by hemorrhage or other abnormal condition.

The record above discussed and many similar tracings support the proposition advanced by Erlanger that the pulse-pressure (the difference between systolic and diastolic arterial pressure) is proportional to the systolic volume discharge of the heart. The present writer believes, however, that this proposition will require certain qualifications. As further work in this field is promised by Erlanger, the matter will not here be discussed further.

The Force which Fills the Ventricles.

Whether the filling of the ventricles is wholly dependent on venous pressure, or is due to venous pressure aided by a suction developed within the expanding ventricle, is a question which logically demands discussion in this place.[2] Yet, as the question is one of considerable difficulty and complexity, this discussion may properly be left for a later paper. Only the conclusion to which the writer has been led will

[1] For literature see Tigerstedt, R.: Ergebnisse der Physiologie, 1903, II, 2, pp. 560–565.

[2] See technic of the pressure curves, page 336, and paragraph 3, page 367.

here be stated; a conclusion based upon the study of many volume curves, upon a careful repetition of the experiments of previous writers, and upon researches also along novel lines.[1] It is as follows:

The blood streaming along the veins toward the heart distends the thin but elastic walls of the cardiac reservoirs, — the auricles. The elasticity of the walls of the auricles, thus brought into play, is the force which during ventricular diastole drives the blood through the auriculo-ventricular orifices, and distends the ventricles as rapidly as their walls relax. If during the first stage of diastole the ventricles exert a suction, the importance of such action as an aid in the filling of the chambers is slight. From the hypothesis of an "active diastole," as postulated by Luciani[2] and Stefani,[3] the present writer wholly dissents, although these investigators based their opinions to a great extent upon data similar in character to those described in this paper. Finally it may be stated that the manner in which the filling of the ventricles is described in this paper is not at variance with the conclusion upon this matter to which Porter was led by measurements of the synchronous pressures in the auricle and ventricle, — although at first sight such may appear to be the case. Porter[4] holds that blood flows into the ventricle from the completion of one systole until the onset of the next. All of the graphic records published by him in support of this opinion exhibit a rapid rhythm of heart-beat. The volume curves in this paper show concordantly that, when the rhythm of beat is rapid, the filling of the ventricles begins at the completion of one systole and ends at the onset of that succeeding. They show that it is only when the rhythm is slow that the filling of the ventricles is completed in a period considerably less than that elapsing from the termination of one systole to the onset of the next.

The Relation of the Amplitude of the Volume Curve to the Rate of Beat.

The volume curve, when the rate of heart-beat is slow, may be described roughly as consisting of a down stroke, an up stroke, and a horizontal line. These lines correspond to the contraction, relax-

[1] Conclusions similar to these, and based apparently on similar data are reported by R. von den Velden, Zentralblatt für Physiologie, 1906, xx, p. 73.

[2] See page 330 of this paper.

[3] See page 332 of this paper.

[4] See page 329 of this paper.

ation, and rest of the cardiac muscle. They express the discharge of the ventricular chambers, their refilling, and the interval elapsing before the next discharge. Variations in the rate of heart-beat are effected chiefly by varying the length of the interval of rest. Within the limits of the normal variations in the rate of beat the down stroke or discharge and the up stroke or refilling remain practically unaltered. Within these limits, therefore, the output of the heart per minute must be proportional to the rates of beat. When, however, the rate becomes so rapid that the interval of rest between relaxation and contraction wholly disappears, the output reaches a maximum. Any further increase in the rate of beat necessarily involves a diminution in the volume discharged by each individual beat. Thus the output, which is the product of the rate and amplitude of beat, remains practically stationary. Indeed, beyond a certain point the diminution in amplitude is more than proportional to any increase in rate.

An example of the graphic records on which these statements are based is reproduced in Fig. 15.[1] The three records show the form of the volume curve at rates of 90, 120, and 200 beats per minute respectively. The first and (for a dog) nearly normal heart rate allows a distinct interval of rest between the completion of the filling of the ventricles and the next discharge. The second by eliminating this interval attains practically the maximum output of this heart. As the difference in the amplitude of the individual beats in the two records is very slight (35 and 33 c.c. respectively), the output per minute indicated is very nearly in the proportion of 90 to 120. The tremendous rate of the first three beats in the third record, however, diminishes the amplitude of each beat from 33 to 21 c.c. The slopes of the up and down strokes of the volume curve remain unaltered. Thus the increase of rate from 120 to 200 does not materially increase the output. Neglecting the coronary circulation, but remembering that these measurements indicate the volume changes of the two ventricles together, we find the output per minute of each ventricle at the rates of the second and third records to be 1995 and 2100 c.c. respectively.

In a communication to the American Physiological Society[2] the writer has expressed the belief that the increase of the rate of heart-beat beyond a

[1] Although this tracing has certain technical defects, it has been selected from among a large number of similar records, because it happens to exemplify a greater number of separate points than any one other record obtained.

[2] This journal, 1906, xv, p. 19.

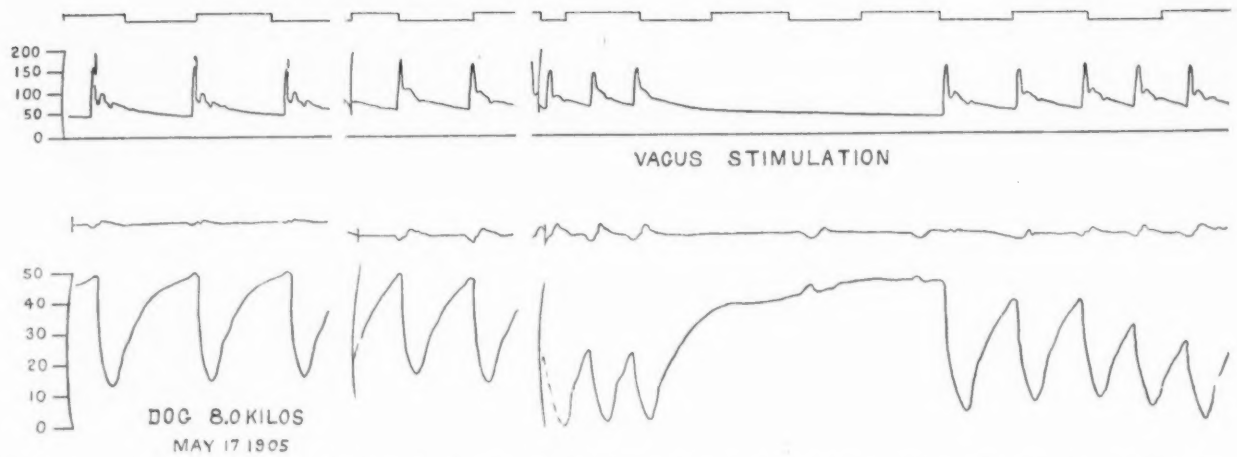


FIGURE 15. — About three-fifths the original size. Time record, aortic pressure, auricular movements, and ventricular volume. In this experiment the natural respiration of compressed air was replaced by artificial respiration. The volume curve in the first record indicates a systolic discharge of 35 c.c. at 90 beats per minute, in the second, 33 c.c. at 120, and in the first three beats of the third, 21 c.c. at 200. Here vagus standstill allows the completion of diastole and a prolonged diastasis, during which the tonus of the ventricles gradually diminishes. This up stroke and the first down stroke thereafter constitute the complete volume curve as discussed in the text.

certain limit involves a more than proportional diminution in amplitude; that the output is thus progressively diminished until finally a condition is reached in which one beat follows another with so short a diastolic interval that the pumping action of the ventricles practically ceases; that such a "tetanus of the heart" is the cause of death in at least one form of shock; and that shock of this form is due to a progressive diminution of the carbon-dioxide content in the blood. The data on which these opinions rest will be presented in a later paper in which will be described also the technic employed to induce the variations in the cardiac rhythm exhibited in the experiments of this paper.

In the third record in Fig. 15 the volume curve goes considerably lower than in the previous records. This indicates that the high rate of beat induces a Treppe involving a more complete emptying of the ventricular chambers than occurs at slower rates. At a rough estimate from the data of these experiments it appears that in a heart beating slowly the discharge volume is only two-thirds of the diastolic volume, — the other third of the diastolic volume remaining in the ventricles at the end of systole. The systolic volume of a rapidly beating heart is on the contrary very small, — the cavities of the ventricular chambers being practically obliterated at the end of systole. Such "staircase" variations do not, however, appear to alter materially the form of the volume curve. They merely determine the level at which the curve occurs. The variations in the form of the curve and its amplitude are apparently determined wholly by the rate of beat.

The Influence of Vagus Stimulation and of Vagus Section upon the Volume Curve.

During the period of ventricular standstill under the influence of vagus stimulation (Fig. 15), the auricles executed two fugitive beats. Their effect on the ventricular volume, as shown in the record, was insignificant. Such records — and many such have been obtained — support the view already expressed as to the dynamic unimportance of auricular systole. They indicate also that in respect to the heart of the dog the vagus in some cases exerts a stronger influence over the ventricle than over the auricle. By a careful adjustment of the strength of the stimulation the writer has frequently (although not invariably) succeeded in bringing the ventricles to complete standstill without alteration (or with only a slight slowing) of the auricular

beat. This result does not, however, necessitate the recognition of direct connections of vagus fibres with the ventricle. It is more probable that it is due to a blocking of the waves of excitation in the auriculo-ventricular bundle, — an explanation which serves also for the occurrence of a considerable lengthening of the period between the end of auricular systole and the onset of ventricular systole on moderate vagus stimulation.

In this connection mention must be made of an observation which has repeatedly occurred in these experiments. On section of both

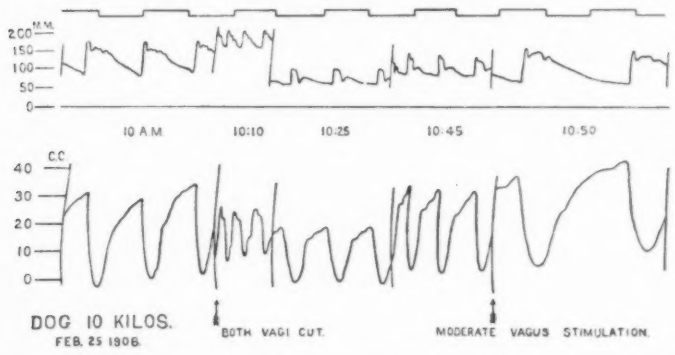


FIGURE 16. — One-half the original size. Time record (added after the other records), aortic pressure, and ventricular volume curves. The volume curve exhibits the sub-maximal character of the beats after both vagi are cut (as at 10.10). Thus, at 10.25 there occur distinct intervals of diastasis, although the beats are of less than two-thirds the normal (maximal) amplitude. At 10.45 the beats have returned so nearly to the normal amplitude that moderate vagus stimulation induces slow maximal beats (10.50).

vagi (or occasionally on section even of one vagus) the heart not only beats more rapidly, but also sub-maximally, — the amplitude of the volume curve being diminished to an extent considerably more than would be the case coincident with the same increase in rate prior to vagus section (Fig. 16). From this observation it would appear that the vagus normally plays a part in holding in check each cardiac excitation until it has attained the strength of a maximal stimulus. The sudden withdrawal of this factor upsets the balance of influences which, normally maintaining the "all or none" character of the heart-beat, allows only of variations in rhythm. In the course of an hour after vagus section, this balance appears to be readjusted to the changed conditions, for the amplitude of the heart-beat as revealed

in the volume curve again becomes maximal. It is important to note that this departure from the "all or none law," and from the principles discussed below, occurs only when the heart just prior to the vagus section has been beating at a slow normal rhythm. This observation appears to support the view presented in the latter part of this paper that the influence of the vagus is not a factor in the development (or in checking the development) of the cardiac excitation, but is a factor in checking the discharge of excitation which induces the heart-beat.

The Principles Underlying the Normal Variations in the Behavior of the Ventricles.

The volume curves obtained at different rates of heart-beat, as in the three records of Fig. 15, appear at first sight to differ widely one from another. On more careful comparison, however, these differences are found to be merely the immediate results of the variations in rate. The individual contraction and relaxation curves at every rate are identical with those at every other rate, so far as the rate allows time for their development. This identity is readily demonstrated. Lay a piece of transparent paper over the first contraction curve following vagus standstill in the third record of Fig. 15, and trace its form from extreme distention down to the point of extreme discharge. Then place the latter point in the tracing upon the corresponding point of the last contraction previous to the vagus standstill, and trace up along the full sweep of the relaxation throughout the period of vagus stimulation. The result is a more complete contraction and relaxation curve than any other recorded. If now this more complete volume curve be superimposed upon every other volume curve of this heart in such a way that the points of extreme systolic contraction correspond, it is found that every point in the latter lies in the more complete curve. It thus appears that the ventricles contract and refill in a manner which is invariable at every rhythm of beat, from the slowest to the most rapid.

The form of this more complete volume curve expresses the most important properties of the heart. The gradient of the down stroke expresses the volume of blood discharged through the semilunar valves during each hundredth of a second that the ventricles are in systole. This gradient is throughout the greater part of the contraction remarkably uniform. When allowance is made for the fact that the stylus of the recording tambour itself moves in the arc of a circle,

the contraction curve becomes at all points, except its extreme lower end, a line which may be treated as straight. This indicates that throughout the greater part of this period the rate of discharge is uniform. The relative volume discharge of every systole, as compared with every other systole, is therefore proportional to the duration of the period of uniform discharge. Since within the limits of the normal variations in rhythm of beat this period varies very slightly, the systolic discharge volume of any heart must be subject to very slight variations. Indeed, we may consider that the output per minute of any heart is proportional to its rate of beat up to that rate at which the duration of systole begins to be distinctly abbreviated.

The gradient of the up stroke expresses the rapidity of the relaxation of the ventricles. The more rapid the relaxation during the early part of diastole the higher the rate of beat attainable at which the duration of systole will not be abbreviated nor its discharge volume diminished. Furthermore, if the complete volume curve is an invariable characteristic for each individual heart, as is here assumed, the form of the complete relaxation curve determines for that heart the duration of systole and of diastole (plus diastasis) at all possible rates of beat. Conversely also it follows from this assumption that the form of this curve may be plotted from measurements of the duration of these periods in the pulse curve. The duration of diastole (plus diastasis) at various rates of beat must be proportional to the abscissal values of points in the complete relaxation curve for which the corresponding ordinatal values must be proportional to the duration of the period of systolic discharge at each of these rates respectively; for the amplitude of every relaxation fills the measure of the next succeeding contraction; and the amplitude of the contractions are approximately proportional to their duration.

The Heart-beat a Twitch Contraction.

In order to appreciate the points which must now be considered, it is advisable to turn the volume curves upside down, so that the contraction and relaxation bear the same relation to the abscissa as is customary in records of striated muscle. When the records are examined in this way, a striking similarity between the volume curve of the ventricles of the mammalian heart and the familiar contraction curves of a muscle nerve preparation becomes immediately apparent. Indeed a little reflection shows us that we are here dealing not merely

with a similarity but with an identity. The volume curve is the contraction curve of a muscle. The variations in the volume curve represent variations in the behavior of the muscle of the heart identical with those of a skeletal muscle under similar conditions in respect to stimuli and load. Thus in Figs. 9 and 10, in which the rate at which the contractions of the heart succeed one another is slow, the volume curve is merely a series of isotonic (or more precisely after-loaded extension) curves, such as are induced by a slow series of single and instantaneous maximal stimuli. In Figs. 11 and 12, the period between successive stimuli shortens during inspiration, so that the time allowed for the relaxations of the muscle of the ventricle is not quite sufficient for it to return to its resting length. In Fig. 14, the interval between stimuli during inspiration is shortened, so that the volume curve takes the form commonly denominated as the "genesis of tetanus." Here, too, the Treppe induced by the rapidly succeeding stimuli is as apparent as in tracings obtained from the gastrocnemius of a frog. Only in one respect does this cardiac "genesis of tetanus" differ from the tracings which the note-book of every medical student contains. It shows no evidence of fatigue; for every relaxation of the heart, even after hundreds of such rapidly succeeding contractions, returns toward the abscissa along the uniform path of the complete relaxation curve. For this the rapidity of the chemical exchanges allowed by the relatively great volume of blood pouring through the coronary circulation affords a sufficient reason. Finally, if we examine the third record of Fig. 15, and ask what it is that causes the great lengthening of the muscle fibres of the ventricle and their subsequent prolonged quiescence during vagus stimulation, it is evident that the simple and complete answer is that during this period the muscle receives no stimuli.

The Ventricle as a Work-adder.

The observation that the volume curve of the ventricles has the same form as the contraction curve of a muscle, leads to the conclusion that throughout the cardiac cycle the volume changes of the ventricular chambers are proportional to the corresponding changes in the length of the muscle fibres in their walls.[1] The relation be-

[1] Strips of heart muscle appear (according to the graphic records published by a number of investigators) to yield isotonic curves essentially similar in contour to those of striated muscle. See, for instance, the records of Erlanger on pp. 176–182 of this volume.

tween volume and extension is similar to the relation between the volume of the cylinder of a pump and the movements of its plunger. The volume of a bag like the heart might be expected to vary not directly, but as the cube of the linear extension of the material composing its walls. The cause of the direct proportionality must be looked for in the arrangement of the fibres of the myocardium. Its attainment affords perhaps the reason for the complex arrangement of the fibres.

The existence of this direct proportionality renders simple the technic of experiments which demonstrate the similarity in the mechanical properties of cardiac and skeletal muscle. The variations in the linear extension and tension of a muscle, when subjected to conditions similar to those under which cardiac muscle works, must be found to be essentially similar to the volume and pressure curves of the ventricles. The analogies between the volume curve and the isotonic curve, the pressure curve and the isometric curve, as described by Frank, have been quoted earlier in this paper. The similarity between the volume curve of a rapid series of beats and that of the "genesis of tetanus" has been discussed. A more rigorous test of the similarity of the properties of cardiac and skeletal muscle may now be described.

The muscle of the ventricles contracts and relaxes under mechanical conditions practically identical with those to which a striated muscle is subjected, when attached to the instrument known as a work-adder. In order to establish the validity of the thesis that the mechanical properties of cardiac and skeletal muscle are essentially the same, it must be demonstrated that the latter, when attached to a work-adder and properly stimulated, behaves as does the normally beating heart. The rhythm of discharge of successive stimuli must be slow. Each stimulus must be single, instantaneous, and maximal. Under these conditions the curves of the tension and extension of the muscle should be found to be similar in form to the ventricular pressure and volume curves; and these curves should bear the same chronological relations to each other and to the movements of the ratchets of the work-adder that the curves from the heart bear to each other and to the movements of the heart valves.

In Fig. 17 are reproduced the tension and extension curves obtained from the gastrocnemius muscle of a frog, working under the conditions shown in Fig. 18. The origin of the muscle is attached to the spring of a Hürthle manometer by means of a lever. The stylus of the manometer records

the changes of tension on the muscle fibres. The Achilles tendon is attached to the lever of the work-adder, so that this lever records the changes in the length of the muscle. One ratchet (*A.V.*) upon the wheel of the work-adder performs a function similar to that of the auriculo-ventricular valves. The other ratchet (*S.L.*) supports the load while the muscle is relaxing, — just as the semilunar valves support arterial pressure during diastole. Above the tension curve are indicated the points

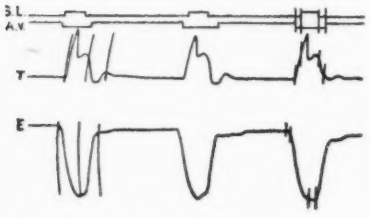


FIGURE 17. — One-half the original size *T*, the tension, and *E*, the extension curves of the gastrocnemius muscle of a frog contracting under the conditions shown in Fig. 18. The up and down strokes of the lines *S.L.* and *A.V.* indicate the points of opening and closing, respectively, of the ratchets corresponding to the heart valves. The relations of these events to the tension and extension are indicated by the synchronous lines on the third "beat."

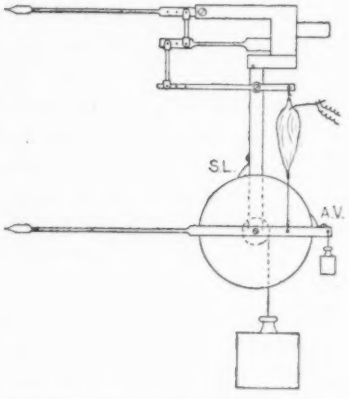


FIGURE 18. — The upper part of the apparatus here represented consists of the spring and stylus of a Hürthle manometer and an additional lever, to which the origin of the muscle is attached. Thus the tension of the muscle is recorded. The Achilles tendon is attached to a lever, by which the changes in the length of the muscle are recorded. By means of the ratchet *A.V.* the muscle at each contraction turns a large wheel and thus raises a heavy weight. When the contraction is complete and the muscle begins to relax, the ratchet *S.L.* holds the wheel and supports this weight, while the muscle is extended by the small weight on the lever.

at which these ratchets open and close. The weight hanging from the axle of the wheel corresponds to arterial pressure. The distance through which this weight is lifted at each contraction of the muscle corresponds to the volume of the blood discharged at each systole. The small weight hanging from the lever of the work-adder, so as to extend the muscle to its original length after each contraction, corresponds to the distending force of venous pressure.

Examination of the record shows that the points of opening and closing of the ratchets bear essentially the same relations to the ten-

sion curve as the graphic records obtained by Chauveau from the heart of a horse exhibit between the movements of the cardiac valves and the intraventricular pressure curve.[1] Indeed, the tension and extension curves of the muscle throughout a cycle reveal practically all the characteristics of the pressure and volume curves of a heart whose rate of beat is slow. If the rate at which successive stimuli are applied to the muscle is progressively increased, the total amount of work done is increased at first proportionately (Fig. 19). The load is lifted further per minute, just as the volume of blood discharge is increased proportionately to an increase in the heart rate. When,

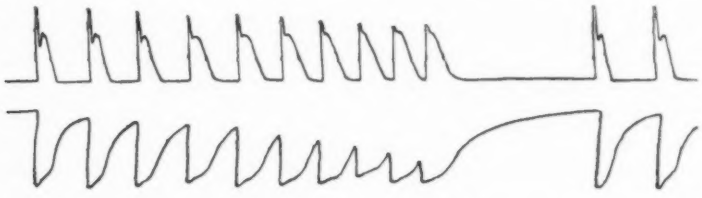


FIGURE 19. — Two-thirds the original size. Tension and extension curves of muscle nerve preparation similar to Fig. 17, but showing also the effects of variations in "rhythm of beat" upon "output" and amplitude. Together with an imitation of vagus standstill.

however, the interval between successive stimuli becomes shorter than the period required for a contraction and complete relaxation, the work done per minute is diminished, just as it has been shown that the output of the heart reaches a maximum, above which maximum an increase in rate causes a diminution in amplitude.

Thus a striated muscle, when working under conditions similar to those to which cardiac muscle is subjected, satisfies the requirements of the principles which have been postulated as controlling the heart, — passing rhythmically through three states, contraction, relaxation, and rest, and exhibiting in its tension and extension curves a behavior identical with that of the heart.

THE PERIODS OF THE CARDIAC CYCLE.

Regarded in this manner the cardiac cycle is seen to consist not only of two periods, but of three; for the heart when beating at a slow natural rate passes rhythmically through three states.

[1] CHAUVEAU, A : Journal de physiologie et de pathologie, 1899, i, p. 377.

1. Systole, the period of the contraction of the ventricular muscle fibres including,

a. The brief interval of rapidly rising tension prior to the opening of the semilunar valves, and

b. The period of discharge lasting from the opening to the reclosing of the semilunar valves.

2. Diastole, the period of the relaxation of the ventricular muscle, including,

a. The brief interval of rapidly falling tension beginning at the instant of closure of the semilunar valves and ending with the opening of the auriculo-ventricular valves, and

b. The period of the refilling of the ventricular chambers beginning at the opening of the auricular valves and ending gradually as the muscle ceases to relax.[1]

3. Diastasis, the period of quiescence beginning gradually as diastole ceases and ending abruptly at the onset of the next systole. During this period there may be no further relaxation of the ventricles if the number of beats executed in the few seconds preceding has been few, and the tonus is therefore slight. If, however, the beats have been occurring in rapid succession, there may be a gradual relaxation throughout this period as the tonus thus induced gradually wears off. This period is of extremely variable duration. Stimulation of the vagus allows the ventricle to come to rest, not in diastole (as the term is here used), but in diastasis. Increase of rapidity of beat up to the point of maximal output is accomplished almost entirely by the abbreviation of this period. At the rate of maximal output, systole follows diastole immediately, and there is no diastasis. With respect to auricular systole it will be evident that, since it always immediately precedes ventricular systole, it may occur during the latter part of ventricular diastasis when the rate of beat is slow; during the latter part of diastole when the rate is that of the maximal output, or even immediately after the preceding ventricular systole when the rate is very abnormally rapid.

The Properties of Cardiac Muscle, the Functions of the Nerve Net, and the Nature of the Vagus Influence.

We may now consider briefly the bearing of the data and discussion above presented upon the question of the cause and nature of

[1] Up to this point this division of the cardiac cycle is essentially the same as that proposed by Hürthle, *loc. cit.*, p. 84.

the heart-beat. Without attempting to review the ever-increasing complexity, to which the myogenic hypothesis has been developed, we may ask, Does the explanation of the behavior of the mammalian heart, as revealed in the volume curve, involve fewer and simpler assumptions if we adhere to the myogenic, than if we employ the neurogenic hypothesis? To this question the writer feels constrained to answer in the negative. Indeed, the analogy which has been drawn between the behavior of the heart and that of a muscle attached to a work-adder and reacting to maximal stimuli discharged by apparatus separate from the muscle, seems almost of necessity (since like results seldom follow from any but like causes) to involve a recognition of a separation of functions in the heart similar to that between the muscle and the stimulating apparatus. Yet the distinction between the two hypotheses in regard to the heart-beat lies in the recognition or rejection of the idea that the machinery which generates the cardiac stimuli is distinct from that which reacts to the stimuli; and in the recognition or rejection of the view that the influence of the vagus is primarily exerted not directly upon the cardiac muscle, but upon the stimulating mechanism contained in the cardiac plexus.

The standstill of the ventricle under the influence of vagus stimulation has been defined above as "a period during which the muscle fibres of the ventricles receive no stimuli." The slowing of the beat by more moderate stimulation is merely a "lengthening of the periods between stimuli," — at least this is the explanation of the changes in the volume curve which would be given by any physiologist who was shown such a record as the lower tracing in Fig. 15, and who — knowing nothing more of it than that it expressed a series of contractions of a muscle — was asked to explain its significance. The volume curves afford, therefore, no support to the idea that the vagus is a "diastolic nerve" in the sense (as held by Stefani) that "it actively induces an increase in the cardiac volume";[1] nor to the view that the vagus exerts a direct regulative influence on the tonus of the ventricles; nor that the vagus "directly promotes the anabolic or assimilatory processes in the cardiac muscle," as held by Gaskell.[2] On the contrary the behavior of the ventricle of the dog's heart, as expressed in the volume curve, is identical with that of the gastroc-

[1] See EBSTEIN'S review, *loc. cit*, p. 182.

[2] GASKELL, W. H.: SCHAEFER'S Text-book of physiology, 1900, ii, p. 221.

nemius muscle of a frog, when the stimuli applied to the muscle are instantaneous and maximal. In the reactions of the muscle under these conditions all variations are dependent on one fundamental variable, — the rate at which stimuli are applied. All similar variations in the behavior of the heart may be explained as dependent on one fundamental variable, — the rate of beat. All the variations in the behavior of the normal heart, induced by the vagus, appear to be readily explainable as due to a lengthening of the periods between stimuli.

The simplicity of this explanation of the underlying reasons for the variations in the behavior of the mammalian heart, is in marked contrast to the complexity of those to which Gaskell, Engelmann, and other supporters of the myogenic theory of the heart-beat have been led by the study principally of the frog's heart. Thus Gaskell holds that in respect to "the primary action of the vagus on the heart of the cold-blooded animals, . . . the inhibitory action is a direct one of nerve fibre upon muscle fibre, and affects all the properties of the muscle fibre in the same way — rhythmicity, excitability, conductivity, contraction-force, and tonicity."[1] Engelmann[2] goes even further and postulates the existence in the vagus of distinct sets of nerve fibres controlling respectively the chronotropic, bathmotropic, dromotropic, and inotropic variations, which he finds in the frog's heart. The volume curves presented in this paper show that the mammalian heart also varies in rhythmicity, tonus, and contraction-force (or amplitude). When the respiratory variations are prominent, the interval between auricular and ventricular systole also exhibits variations which indicate differences of conductivity. Fig. 15 and the textual remarks upon it indicate variations in excitability. Yet all these phenomena, at least as they appear in the mammalian heart, admit of ready explanation in the simplest manner if we relinquish the myogenic theory, and postulate for cardiac muscle only such properties as are possessed by striated muscle. Adopting the neurogenic hypothesis we may consider that the variations in the behavior of the muscle of the heart, exhibited in the volume curve, indicate processes in the nerve net resulting in discharges of stimuli into the muscle similar to those which when applied to striated muscle produce a similar behavior. We may then employ a muscle nerve preparation as an instrument from whose reactions under known con-

[1] GASKELL, W. H.: *Loc. cit.*, p. 215.

[2] ENGELMANN, TH. W.: Archiv für Physiologie, 1900, p. 315.

ditions we may infer similar conditions in respect to the stimuli discharged into the cardiac musculature, — in other words, the properties of the cardiac plexus. The first conclusion to which this line of reasoning leads is that the plexus normally discharges only maximal stimuli; for every contraction is maximal; and since tonus at every instant is proportional to the number of beats in the period immediately preceding, we must infer an entire absence of those subminimal stimuli which induce tone in a resting skeletal muscle. The statement that the plexus of the mammalian heart normally discharges only maximal stimuli, must not be understood to mean that the plexus is essentially incapable of submaximal discharges. On the contrary, when the mammalian heart ceases to beat vigorously with complete co-ordination and uniform rhythm, its volume curve may exhibit a wide diversity of forms. Such curves resemble the contraction curves of striated muscle induced by stimuli of irregular series and submaximal strength. They may be explained accordingly as due to irregular and submaximal discharges by the cardiac plexus. Regarded thus they strengthen the view that cardiac muscle is merely a somewhat modified variety of striated muscle, and that the behavior of each is equally the expression of the stimuli discharged into its fibres through nervous channels. The essential difference in the behavior of the heart and that of a skeletal muscle in the living body lies in this, — that the contractions of the latter usually occur under the influence of a very rapid series of submaximal stimuli. They are tetani of greater or less duration and strength. The beat of the heart, on the contrary, is a "twitch" contraction induced by a single and nearly instantaneous stimulation.

To the cardiac plexus we must accordingly assign the property of effecting a continual algebraic summation of various stimuli, chemical (such as oxygen, the Na, K, and Ca ions, etc.), mechanical (from the coronary and venous pressures), and nervous (from vagi and accelerators) in such a manner that the excited state of each unit of the plexus is not discharged into the muscle until it has swollen to the proportion of a maximal stimulus. Indeed, by utilizing the modern view of the properties of the synapse, we may explain the all or none character of the heart-beat by assuming that, as compared with the interneuronic connections in the central nervous system, the junctions of the nerve units composing the cardiac plexus are of high resistance. Normally they yield no passage to any excitation of an intensity less than that which will induce a maximal contraction in

the cardiac muscle. When, however, the pressure of excitation becomes sufficient to break through the junction, the passage is wholly free and complete.

Furthermore, the entire range of vagus influence appears to be explainable on this view by the assumption that all its fibres belong to a single class, and that their function is to increase, or under uniform normal conditions to assist in maintaining, the resistance of these junctions.[1] This assumption accounts for the immediate results of the section of both vagi previously described. It accounts for the slowing of a normal rhythm without increase of amplitude of beat; for while under such conditions the excitations must be dammed back to a supermaximal intensity, supermaximal stimuli can induce in muscle only maximal contraction. It accounts for the increased amplitude which accompanies slowing, when the functional activity of the plexus is below normal and its discharges are therefore submaximal. Finally, it accounts for the variations induced by vagus stimulation in rhythmicity, excitability, and conductivity. Tonus and amplitude of beat (both in respect to Treppe and to genesis of tetanus) have already been explained, at least for the normal heart, as the reactions of the cardiac muscle to the rhythm of stimulation.

Conclusion.

Since the ventricles can discharge at each systole only so much blood as enters their chambers during diastole, the principles controlling and the conditions determining the diastolic filling of the ventricles control and determine the variations in the volume of the blood which is discharged into the arteries at each heart-beat. Present knowledge of this subject, so far as it is based upon observation and experiment upon the mammalian heart, is not sufficient to afford a clear and complete explanation of these principles and conditions. Present teachings indeed consist largely in an immediate application of data obtained from the frog's heart to the explanation of the mammalian circulation. The study of the frog's heart has shown that apart from variations in arterial pressure and rhythm of beat "the amount of the heart work is capable of being varied within wide limits by variations in the venous pressure, and more so still by

[1] For a similar view in respect to the vagus influence in the frog, see Muskens, L. J. J.: This journal, 1898, i, p. 510. For a study of the vagus and accelerator influences on the mammalian heart, see Hunt, R.: This journal, 1899, ii, p. 395.

variations in the force of the auricular contractions." Indeed, in the frog it appears that "it is through its influence on the auricle, which must be looked upon as a much more highly developed organ than the ventricle, that the nervous system regulates the work done by the heart."[1]

It appears from the data presented in this paper that these statements do not hold true for the mammalian heart under conditions altered from the normal no further than the opening of the thorax. While the filling of the ventricles of the mammalian heart will of course be interfered with by any condition which interferes with the flow of the blood along the veins toward the heart (such as extensive hemorrhage or choking), the extent of filling of the ventricles is not appreciably varied by variations in venous supply or pressure within normal limits. Auricular systole plays a part which is mechanically of very minor importance in the normally beating heart. The mechanical function of the auricles of the mammalian heart appears to be more nearly analogous to that of the sinus venosus than to that of the auricles of the frog. The mammalian auricles are to be regarded as elastic reservoirs rather than as force pumps. When the dog's heart is beating at a slow natural rhythm the ventricles are refilled so rapidly after each discharge that a period of quiescence may intervene after the refilling is completed, and before the onset of auricular systole. Under such conditions the auricles inject only a small fraction of a cubic centimetre of blood into the already distended ventricles. The currents in the blood thus induced may cause the closure of the auriculo-ventricular valves. Except for this possible effect auricular systole is a factor of almost negligible dynamic value.

The vagus exerts one fundamental influence upon the mechanics of the mammalian heart when beating with normal vigor. It slows the rate of beat. All other mechanical variations in the pumping action of the heart are derivatives of this fundamental. Thus the influence of the vagus upon the auricles is important only as alterations of auricular rhythm induce corresponding alterations in ventricular rhythm. The vagus indeed exerts (indirectly) an influence upon the ventricle, stronger than that upon the auricle; — for in these experiments it has repeatedly (although not invariably) been found possible, by a nicely adjusted strength of stimulus, to bring the ventricle into standstill without altering, or with only a slight slowing of

[1] Roy, C. S.: Journal of physiology, 1879, i, pp. 470, 496.

the auricular rhythm. Section of both vagi, or sometimes even of one vagus when the rhythm of beat prior to the section was slow, leaves the heart for a time in a condition in which it not only beats more rapidly, but also submaximally. No such departure from the "all or none" behavior of the heart occurs if the rate of beat prior to the section was very rapid. This difference indicates that the vagus influence is not a factor in the processes by which the cardiac excitation is developed, but that it is a factor in regulating the intensity to which the excitation must be developed before it becomes sufficient to discharge into the contractile tissue. These observations indicate a distinction between the mechanism in which the excitation is developed and the mechanism reacting to the discharge of the excitation, and accord therefore with the requirements of the neurogenic hypothesis.

The cardiometer described in this paper, while not free from theoretical objections and technical defects, affords an easy method of studying the volume changes of the ventricles. It has one essential advantage over other forms of cardiometer, and over the use of the pericardial sack as the cardiometer chamber, in that only the ventricles are enclosed. It yields the volume curve of the ventricles alone. This volume curve closely resembles in form an isotonic or afterloaded muscle curve. It indicates that the filling of the ventricles proceeds as rapidly as the relaxation of the ventricular muscle allows. As the relaxation is rapid at first and toward completion more gradual, so the filling of the ventricles is rapid at first, immediately after the completion of systole, then more gradual. The period of relaxation or filling bears the same chronological relation to the period of contraction or discharge that the relaxation of a striated muscle bears to its contraction. It has been found that the volume of the ventricular chambers varies directly proportionately to the extension of the cardiac muscle fibres in their walls. The similarity of the heart-beat to the maximal twitch contraction of a skeletal muscle is attested by the similarity between the volume and pressure curves of the ventricles and the extension and tension curves of a frog's gastrocnemius attached to a work-adder. On this similarity are based the following conclusions:

(1) The cardiac cycle consists not only of two periods, but of three. Systole, the period of contraction and discharge; diastole, the period of relaxation and refilling; diastasis, the period of rest.

(2) Variations in rhythm of beat up to a certain optimum rate are

brought about almost entirely by variations in the duration of diastasis. At all rates below this optimum, the volume discharge of all beats is nearly uniform. Up to the optimum rate the output of the ventricles per minute varies almost directly in proportion with the rate. At the optimum rate no period of diastasis occurs; systole follows immediately upon diastole. Increase of rapidity of rhythm above this rate involves a shortening of systole, a diminution of the volume discharge of each beat, and no further increase in output. This behavior of the ventricles is similar to that of an excised muscle, as represented in curves of superimposed contractions, and genesis of tetanus.

(3) The systolic discharge volume of normal heart beats is approximately 60 per cent of the total volume difference between extreme relaxation (as after prolonged vagus standstill) and extreme contraction (as exhibited in hearts which at autopsy are found "in systole"). At slow normal rhythms the ventricles do not relax wholly after each beat; for during vagus standstill, as the tonus of the muscle wears off, their volume is gradually increased to the extent of an additional 10 or 15 per cent. Nor at such rhythms are their chambers emptied by systole; from 20 to 30 per cent of their diastolic volume is retained, for the volume of the ventricles is further diminished to this extent by the development of the "cardiac tetanus" above mentioned. In respect to the cardiac muscle it appears that both the diastolic contraction remainder (tonus) and the systolic contraction excess (Treppe) at the onset and end of each beat respectively depend in nearly equal degree upon the rhythm of the beats during the few seconds preceding. In other words, the increase of tonus with each increase of rate up to the optimum rate appears to diminish the diastolic volume of the ventricles by amounts equal to — or slightly greater than — the amounts by which the coincident development of Treppe diminishes their systolic volume. The amplitude of beat or systolic discharge volume is therefore nearly uniform at all suboptimal rates, while such variations as do occur tend toward a diminution of the amplitude of beat at high as compared with low rates.

(4) The contraction and relaxation curves at every rate are identical with those at every other rate so far as the rate allows time for their development. The manner in which the ventricles contract and refill, as expressed in the "complete contraction" and "complete relaxation" curves, is for any heart an invariable individual characteristic. The form of this complete volume curve determines the dura-

tion of systole and of diastole (plus diastasis) at all possible rates of beat.

(5) The explanation of the behavior of the heart, as revealed in the volume curve, upon the analogy of similar curves obtained from a muscle nerve preparation, leads the writer to the conclusion that the simplest explanation of this behavior is found in the neurogenic hypothesis of the heart-beat. According to the explanation here offered, cardiac muscle possesses those properties and those only which are possessed by ordinary striated muscle. The properties of the cardiac plexus are such as result in the discharge of stimuli of a character similar to those which induce in a skeletal muscle a behavior similar to that of the heart. This explanation involves the assumption of only one set of fibres in the vagus, — all of identical character, — whose function is that of regulating the resistance offered by the junctions of the nerve units composing the cardiac plexus to the transmission of excitation.

[ADDENDUM. — The principal argument of this paper is based upon the assumption that the isotonic curve of cardiac muscle is similar in form to that of skeletal muscle. That such is the case in respect to mammalian cardiac muscle has, however, been denied by Fredericq,[1] who holds that the contraction curve of this tissue has a trapezoidal form similar to that of the intraventricular pressure curve. While this objection to our argument might be met with the suggestion that the method employed by Fredericq may have recorded the tension rather than the extension of the cardiac muscle (as in records of the apex beat in man), we are inclined to believe that this is not the principal explanation of the curves obtained by Fredericq. We have observed that skeletal muscle (gastrocnemius of frog) also yields trapezoidal or contracture curves when working under mechanical conditions similar to those to which the cardiac muscle was subjected in Fredericq's experiments. If the small extending weight on the lever of the work-adder to which a frog's gastrocnemius is attached (as shown in Fig. 18) be so reduced as barely to re-extend the muscle a few contractions may transform the normal extension curve into a typical contracture curve, or even induce a permanent contracture from which the muscle does not recover its extensibility. A tendency toward the trapezoidal form is exhibited in the extension curves of Fig. 19.

Furthermore we find that the reason that the left ventricle of the heart of a cat or dog under perfusion with Locke's fluid frequently fails to beat, while the remainder of the heart beats vigorously, lies in a contracture due to the absence of any distending force within the ventricular chamber. If under

[1] FREDERICQ, L.: Archiv internationales de physiologie, 1906, iii, p. 385.

these conditions the left ventricle be distended with fluid supplied through the auricle under a few centimetres' head of pressure, this part of the heart begins to beat vigorously. We have repeatedly seen cats' hearts in which the left ventricle under this treatment recovered so completely that within a minute it was pumping normal volumes against a normal arterial pressure (150 cms. of Locke's fluid). Within two minutes after the supply of fluid to the auricle was stopped the left ventricle passed again into complete contracture stand-still, although the perfusion of the coronary vessels from the aorta continued unaltered. We conclude therefore:

(1) That the contracture curves obtained by Fredericq are due to the fact that in his experiments the heart was perfused but not distended.

(2) That the similarity of the contracture developed by mammalian cardiac muscle and that induced in a skeletal muscle by similar mechanical conditions is an additional indication of the fundamental identity in the properties of these two varieties of muscle.

(3) That any real suction pump action by the left ventricle is impossible, for the lack of a pressure in the auricle sufficient to distend the ventricle results in a contracture of the ventricular muscle. In further support of this conclusion it may be stated that we have performed three experiments (using the general method described in the foregoing paper) in which a pressure was exerted upon the ventricle of the heart of a dog by forcing air into the cardiometer. When the pressure upon the ventricle was raised to within 3 mm. of mercury of the venous pressure at the same instant, the pumping action of the heart was practically abolished, — as was indicated by the sudden fall of arterial pressure.]

A REFLEX RESPIRATORY CENTRE.

By IDA H. HYDE.

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Introduction.

THE attempts to determine whether the respiratory centre in mammals is automatic or reflex in character have been fraught with insurmountable difficulties, owing to the impossibility of isolating intact, that centre from all others, and then studying its nature. But in Limulus, as I have shown,[1] the respiratory mechanism is governed by the branchial ganglia of the ventral nerve cord, and each ganglion which is a centre for the activities of its segment can be isolated from the nervous system, or can be sectioned into two lateral halves without interrupting the respiratory movements of the particular segment or lateral half to which it belongs. Consequently, we possess in Limulus most favorable conditions for the investigation of the automatic or reflex character of the respiratory centre, as well as rare opportunity to study upon that isolated centre the effects of gases, temperature, drugs, and other chemical solutions. During the present investigation, a study of the influence of some of these factors upon the rhythmical respiratory activity was begun. The work was undertaken at the Marine Biological Laboratory in Woods Hole, where it is planned soon to pursue it farther.

The paired branchial ganglia of the ventral nerve cord are the parts that especially concern us and merit description. Fig. 1 represents a ventral view of the principal parts of the nervous system, and Fig. 2 the nerves connected with a branchial ganglion. Dissection as well as mechanical and electrical stimulations corroborate Patten and Redenbaugh's[2] description of these. The ventral nerve cord consists of three separate paired branchial ganglia, and posterior

[1] Hyde, I. H.: Journal of morphology, 1894, ix, p. 436.

[2] Patten and Redenbaugh: Journal of morphology, 1900, xvi, p. 94.

to these, three intimately united ganglia connected by a double nerve cord and transverse commissures. Each ganglion has an afferent anterior and a mixed posterior nerve root. The anterior root consists of four important branches that conduct impulses to the ganglia from the longitudinal abdominal muscles, intestinal nerve plexus, cardiac plexus, and the carapace. I was surprised to find that each of these branches contains both accelerating and inhibitory respiratory fibres, while the posterior root supplies both the sensory and motor fibres for the branchial muscles and the gills. The respiratory movements consist in a consecutive dropping and raising of the gill-plates, respectively known as the inspiratory and expiratory phase. During the former, the gill-books become fully dilated and their contents of blood thoroughly exposed to the water, while in the expiratory phase the gill-books are flattened and their contents forced out. The respiratory movements are very variable, depending upon the conditions of the external medium and the internal state of the animal.

Since the gill-plates are modified swimmerets, they are active in all forms of locomotory movements, and although they may be inactive when the animal is at rest, they more often are executing regular rhythmical respiratory movements. Moreover, in normal animals, after stimulating certain parts of the nervous system, peculiar cramp-like movements of the gill-plates occasionally make their appearance. A most remarkable fact observed during this investigation was, that the respiratory activity of the gill-plates continued in some instances as long as forty hours, not only when the heart but when most of the blood as well as the heart was removed from the body. If the intestine was also cut out, the respiratory movements did not continue so long. In fact, as shall be shown later, the rhythmical phases of the gill-plates are not interrupted when their isolated ganglion with its posterior roots is lifted from the body, but their rate and rhythm may differ from those still attached to the ganglia intact with the abdominal cord. Moreover, Limuli can live for many days in air, but the respiratory movements are quite irregular, the gills often remaining for long periods in the inspiratory phase. The composition of the water and its contact with the gills may be in Limulus a constant summation of stimuli like that of blood in higher animals at the centre and may periodically become adequate. The above facts indicate that the respiratory movements in Limulus are to a great degree independent of the gas volume of the blood, and that in bloodless Limuli the vitality

gradually fades away without displaying movements comparable to dyspnœa in higher vertebrates.

Method.

During the operation the animal was fastened on an inclined plane so that the gills were, but the exposed ganglia and nerve roots to be operated on were not, under water, while a constant stream of sea water flowed over the sides and back of the animal. Under these conditions, the gills continued their rhythmical respiratory activity for an indefinite time.

Knowing that each ganglion of the ventral nerve cord or each lateral half of a ganglion can be isolated not only from the brain but from each other without interrupting the respiratory movements of the gill-plates innervated by them, the problem of ascertaining whether the branchial ganglia are automatic or reflex in character seemed at first a simple one. It was but necessary to prevent sensory or afferent impulses from reaching them by a method that avoided shock or stimulating effects, and did not destroy the irritability of the ganglia or the conductivity of the motor nerves. I found that if the animal was partially anæsthetized with ether, and the regions of the cord or nerves that are to be cut treated with cocaine or frozen with ether vapor, that shock and stimulating effects were avoided. It was possible, therefore, to proceed in the process of isolating a respiratory centre as far as cutting the cord anterior and posterior to it, and then all the branches of the anterior root, without interrupting respiration. Since the sensory and motor fibres are so intimately combined in the posterior root it is impossible to cut one set without the other. This fact proved the stumbling-block in the whole experiment. It was necessary, therefore, to devise some method by which the sensory paths could be blocked without affecting the functions of the motor fibres.

For the purpose of paralyzing the sensory fibres without disqualifying the motor, two successful methods were finally adopted. In the one cocaine hydrochlorate and in the other strong electrical currents were employed to block the afferent impulses to the branchial ganglia. The results of many experiments demonstrated that the length of time of action and the strength of the cocaine or electrical current required to paralyze the sensory fibres was not the same for all Limuli. These factors, therefore, had especially to be determined

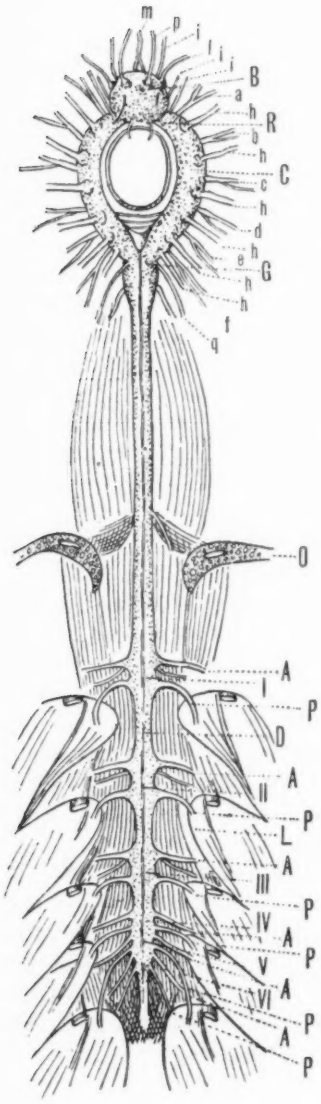

FIGURE 1. — This figure represents a ventral view of the principal part of the nervous system of Limulus, ensheathed by arteries not enlarged from the specimen.

B. Anterior œsophageal ganglion or brain.
C. Œsophageal collar.
G. Post-œsophageal ganglion.
D. Abdominal ganglionic cord.
I–VI. Ganglia of the cord.
A. Anterior ganglionic nerves.
P. Posterior ganglionic nerves.
L. Cut edge of the gill plates.
O. Cut edge of the operculum.
a–f. Integumentary nerves.
h. Nerves to the thoracic appendages.
n. Nerves to the pencils.
q. Nerves to the operculum.
r. Nerves to the chiliceræ.
m. Nerves to the median eye.
p. Nerves to the subfrontal sclerite (olfactory organ).
l. Nerves to the lateral eye.
i. Nerves to the frontal integument.

for each individual animal previous to the operation. A survey of the results of a long succession of experiments proved that cotton fibres soaked in 0.2 per cent to 0.5 per cent cocaine hydrochlorate applied for ten to thirty minutes to the posterior root completely blocked the sensory but not its motor impulses. It required three to twenty minutes more to paralyze the latter.

To achieve the same results with the electrical current, it was necessary to stimulate with strong induction currents for thirty to thirty-five seconds, while the motor impulses were blocked in forty to fifty seconds. As soon as the sensory impulses in the posterior root are cut out, the branchial muscles of that side lose their tone, and mechanical stimuli applied to the innervated region fail to elicit a response either on the same or opposite side. In addition to these manifestations of inhibition or block of sensory impulses, the threshold value of the electrical current was employed to test the efficiency of the block. Before attempting to cut out the afferent impulses, the threshold value of a current applied to a posterio root of an isolated ganglion that would stimulate the branchial muscles of the same side, and also that value which, when applied to the same spot stimulated the movements of the gills on the opposite side, were accurately determined. In the latter case, the impulse passed to the ganglion, and from it in the posterior root on the opposite side to the branchial muscles of its side. With these data at hand, the sensory block was investigated by applying the threshold current to the posterior root peripherad and centrad to the blocked region, and then to the posterior root of the opposite side. If the block was complete, the threshold strength of current applied peripherad to it would cause increased activity of the gill-plates on the same side through motor fibres, but not of those on the opposite side, since the impulses could not reach the ganglion. On the other hand, if that current were applied centrad to the block it would stimulate the gills on the same side through the motor fibres as well as those on the opposite side, and the same strength on the posterior root of the opposite side would produce increased respiratory movements on the blocked side, thus proving altogether the existence of the afferent block in the posterior root of one side. The block in the opposite posterior root is then easily achieved.

Observations and Summary.

Impulses reach the branchial ganglia not only from the gills and branchial muscles through the posterior roots, but also through the

anterior roots, as has been stated above, from the integument, viscera, heart, and abdominal longitudinal muscles that govern the hinge movements of the telson and mesothorax. When the heart is exposed with much loss of blood, the heart and respiratory rhythm continue quite normally. Stimulating under these conditions the lateral cardiac nerves in certain regions, with weak induction shocks, accelerates the heart's action and inhibits respiration. Stimulating, however, in the same region with strong induction shocks may accelerate both

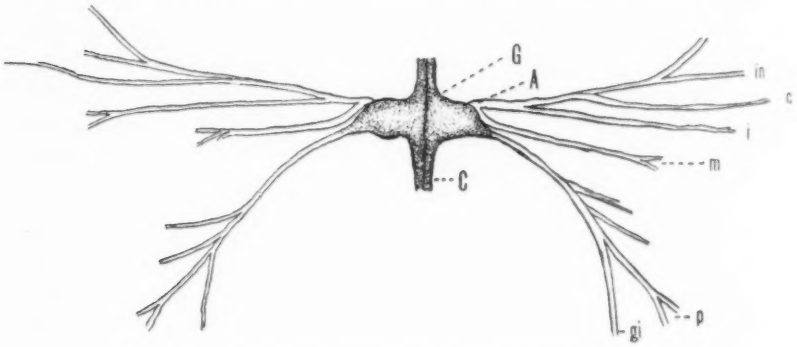

FIGURE 2. — An enlarged ventral view of an abdominal cord ganglion.

A. Anterior root.
C. Cord.
G. Ganglion.
i. Nerve from intestinal plexus.
c. Nerve from cardiac plexus.
in. Nerve from integument.
m. Nerve from longitudinal abdominal muscles.
P. Posterior root.
gi. Nerve from gill-books.

the heart and respiratory movements, while weak induction shocks applied to the cardiac inhibitory fibres arrest the heart, and may in some cases also inhibit the movements of the gill-plates. Whether acceleration or inhibition of respiratory movements follow stimulation of the lateral cardiac nerves, depends upon the strength of the current and also the region stimulated.

When the heart and most of the blood are removed, the gills continue their rhythm, and if now the intestine is detached, the movements become perceptibly weaker. Weak induction shocks applied to the fibres extending from the intestine to the branchial ganglia produce inhibition, but sectioning these fibres causes marked decreased action of the gill-plates on the operated side. Employing the same or stronger currents to larger fibres in the same or other regions of the intestinal plexus, at once causes acceleration of respiratory

activity. I had also observed that when Limulus was being fed, the respiratory movements were always accelerated. The above facts demonstrate that stimuli from the intestine as well as from the heart may accelerate or inhibit the respiratory movements, and that both inhibitory and accelerating fibres extend from these organs to the branchial ganglia.

The respiratory movements moreover are conspicuously associated with the contraction of the flexor and relaxation of the extensor muscles of the mesothorax. A slight touch on this region may instantly cause strong flexion of the mesothorax and cessation of the respiratory movements. If now a stream of water flows over the animal, the mesothorax gradually relaxes and respiratory activity gradually reappears. Direct stimulation of the extensor or flexor muscles or nerves from them to the branchial ganglia invariably produce, respectively, acceleration or inhibition of respiratory activity. When, therefore, the paths from the heart, intestine, longitudinal abdominal muscles, brain, segmental carapace, and neighboring ganglia are completely cut off, there remains only the afferent path to the branchial ganglia in the posterior root, from the integument and muscles of the gill-plates. Under this condition the respiratory movements are quite weak. They can, however, be augmented or inhibited by suitable mechanical or electrical stimulations of the integument of the gill-plates, and accelerated if the posterior roots are stimulated.

For the purpose of studying the nature of the branchial ganglia, it is first necessary, therefore, to bring them to this almost complete state of afferent isolation. From this stage onward, to the achievement of reliable facts, various methods were pursued. I shall briefly outline three of these that led to definite results. According to the first method, the rate and force of the respiratory movements is noted, for instance, of the gill-plates of the second branchial ganglion, isolated from all afferent paths excepting those in the posterior roots from the gill-plates, and then the threshold value of electrical stimulation is determined for its posterior roots, as above described. The character of the response to mechanical and electrical stimuli applied to different parts of the gill-plates is also ascertained. With these data at hand, I proceeded to block, *e. g.*, the left posterior root of the isolated ganglion, either with a 0.3 per cent. solution of cocaine hydrochlorate applied for about twenty minutes, or induction currents for thirty seconds to the median portion of that root. The result was, loss of muscle tone, failure to respond to peripheral mechanical

stimuli and slower almost imperceptible movements of the gill-plate on the blocked side. The only remaining afferent paths to the ganglion are the sensory ones in the posterior root of the opposite side. Stimulating the nerve peripherad or centrad to the block, or the posterior root of the opposite side with the threshold current, still increases the activity of the gill-plate on the blocked side, thus proving the integrity of the motor fibres. But the stronger threshold current when applied peripherad no longer will, but centrad to the block will, accelerate the movements of the gill-plate on the opposite side, demonstrating thereby the efficiency of the sensory block. At this stage the opposite posterior root may be cut or blocked with cocaine, electrical current, or by freezing. In any of these cases, the movements of the gills on both sides cease. By washing off the cocaine the respiratory rate again coincides with that of the gill-plate on the other side. If now the cocaine, or electrical current block, is produced in the posterior roots on each side of the isolated ganglion all possible afferent impulses are cut off, and the ganglion is now in a position to demonstrate its automatic power. But under this condition all movements of its gill-plates cease, accompanied by the phenomena following loss of sensory conduction. Moreover, the mechanical and electrical tests prove the efficiency of the sensory block and that the ganglion and motor fibres are uninjured, and conduct impulses.

In another series of experiments, the ventral nerve cord was cut posterior to, *e. g.*, the second branchial ganglion, its right and left halves were separated by a median incision, and the anterior root of each half was cut; furthermore the ventral cord was cut anterior to the right half, but the left half remained in connection with the cord anteriorly. The gill-plates of each half moved rhythmically, but those on the right much weaker. If cocaine is now applied to both posterior roots, for the previously determined length of time the gill-plates lose their muscle tone, fail to respond to mechanical stimuli and still respond to the threshold current applied peripherad and centrad to the block, but the gill-plate of the right half has stopped, while that of the left half still connected anteriorly with the ventral cord and brain is moving rhythmically. If the cord anterior to the ganglion is now frozen the left gill-plate at once ceases its respiratory activity.

Another modification in the method consisted in cutting the cord posterior to the ganglion and hemisectioning the right half of the cord anterior to the right half of the ganglion. The anterior roots of both sides were cut and the posterior roots of both sides treated with

the previously determined strength of cocaine for about twenty minutes. If the usual tests proved the block efficient, and the gill-plates of both sides still moved, though the left very faintly; since some afferent impulses from the brain could still reach the left half of the ganglion through the ganglionic commissure, then freezing or freezing and cutting the cord anterior to the ganglion produced instant cessation of gill activity. The movements were again resumed, when the freezing or the cocaine blocks were removed, or if after cutting the cord the cocaine was washed off from the posterior roots.

A few experiments were tried to test the influence of the gas contents of the water upon the respiratory movements. Small Limuli were kept in air-tight flasks of sea water through which a constant stream of pure carbon dioxide flowed. In a minute the irritability and excitatory state which resembled the first effect of ether ceased without dyspnœic activity. In all cases the normal respiratory rhythm returned when the animals were placed in fresh sea water even when they were kept in the carbon dioxide water for several hours. That this effect is due to peripheral and not central stimulation is further shown when purified oxygen or pure hydrogen instead of carbon dioxide constantly flowed through the flasks. In the former gas no apnœa and in the latter no dyspnœa was displayed in forty-eight hours, and the slowed movements that then existed in the hydrogen charged water were replaced by a normal rate and rhythm in fresh sea water. These results corroborate Walling's [1] experiments of a similar character, conducted two years ago in my laboratory, on the influence of gases upon the respiratory and heart action in grasshoppers.

The rhythmical respiratory movements in Limulus are not dependent upon the gas volume of the blood, because they persist in bloodless animals. The experiments demonstrate furthermore that the branchial ganglia in Limulus are reflex in character and that the respiratory movements, though they may be influenced by other factors, are dependent upon, and modified by, impulses from the brain, heart, and intestinal plexuses, abdominal longitudinal muscles, peripheral stimuli and conditions of the external medium; especially the gas contents of the water.

According to Gaskell [2] the branchial ganglia in Limulus are comparable to the ganglia that control the movements of the branchial

[1] WALLING, E. V.: Journal of experimental zoölogy, 1906.

[2] GASKELL, W. H.: Brain, 1899, lxxxvii, p. 339.

gills in Ammocœtes and the hagfish, and are homologous to the respiratory centre in higher vertebrates. I showed that in the skate [1] the respiratory centre is segmental in character, illustrating in its type of respiratory centre an intermediate stage between the simple segmental arrangement of neurons, presiding over the co-ordinated respiratory movements found in Limulus, and the complex modified and specialized centres existing in higher vertebrates.

[1] HYDE, J. H.: This journal, 1904, x, p. 236.

ON THE CHEMICAL CONDITIONS FOR THE HEART ACTIVITY, WITH SPECIAL REFERENCE TO THE HEART OF LIMULUS.

By A. J. CARLSON.

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I. Introductory.

THE experiments reported in this paper were undertaken for the purpose of determining the specific influence of the inorganic salts of the blood on the rhythm of the Limulus heart in order to compare the chemical conditions of the automatism of this heart with those of the vertebrate heart. In the Limulus heart the action of solutions on the heart muscle can be studied apart from that on the heart ganglion. It was hoped that a careful determination of the specific action of the blood salts on the two tissues composing the heart might throw some light on the mechanism of action of these salts on hearts in which the nervous and the muscular tissues cannot or have not been isolated for physiological experiments. The specific influence on the Limulus heart of acids, alkalies, and some salts besides those of the blood were determined mainly to test certain theories of chemical stimulation and depression of living tissues.

The work was done at the Woods Hole Laboratory during the summers of 1904 and 1905, the main part during the first season. But the publication of the results has been delayed with the view of testing some possible sources of error in the experiments. In order to conclude that a change in the ganglionic rhythm following the replacing of normal plasma or sea water around the ganglion by plasma or sea water in which the relative proportions of the salts have been altered, — in order to conclude that this change in the rhythm is due to the increase or decrease in the amount of a certain salt it must be shown that the change in the ganglionic activity is not due to possible changes in the osmotic pressure of the two solutions, to

changes in temperature, or to differences in the oxygen contents of the solutions. The influence of these three factors on the ganglion and on the heart muscle has now been determined and the results placed on record. Both the heart ganglion and the heart muscle are very sensitive to changes in temperature,[1] but in these experiments particular care was taken to eliminate the temperature factor by keeping all the solutions at the same temperature.

The relative sensitiveness of the Limulus heart to the lack of oxygen as well as to the presence of carbon dioxide in the medium surrounding the heart was investigated by Dr. Newman under my directions.[2] The heart ganglion as well as the heart muscle exhibits a relatively great degree of independence of the oxygen dissolved in the surrounding fluid or held in combination with the hæmocyanin of the plasma. In fact, the ganglion continues in activity for many hours in the total absence of oxygen in the medium surrounding the ganglion. The possible difference in the oxygen content of the various solutions used in this series of experiments is, therefore, a negligible factor.

In the investigation of the specific influence of osmotic pressure changes it was found that hypertonicity depresses and hypotonicity stimulates both the heart ganglion and the heart muscle.[3] But in these experiments the osmotic pressure factor was eliminated as far as possible by using only isotonic solutions ($\frac{6}{10}\,n$). The specific action of many of the chemicals employed (the acids, the alkalies, the chlorides of barium, potassium, rubidium) must, however, be studied in solutions of less than isotonic concentration, because at that strength they rapidly kill the heart tissues. In order to study the action of these chemicals, their isotonic solutions were mixed with plasma or sea water in any proportion desired. In this way isotonicity is in every case retained at least approximately. The action of chemicals in greater than isotonic concentrations was not studied.

It is evident from the above stated facts that the action of those crystalloids that stimulate the ganglion or the muscle cannot be studied in hypotonic solutions, because hypotonicity itself is a primary stimulant. Crystalloids that depress the heart ganglion or the heart muscle may, however, be studied in hypotonic solutions to some

[1] CARLSON: This journal, 1906, xv, p. 207.
[2] NEWMAN: This journal, 1906, xv, p. 371.
[3] CARLSON: This journal, 1906, xv, p. 357.

extent. For example, if it is found that a $\frac{3}{10}$ *n* solution of calcium chloride or cæsium chloride depresses the ganglionic rhythm or the amplitude of the muscular contractions, it is evident that these chemicals depress, and their depressor action is sufficient to overcome the stimulating action of the hypotonicity. But, if these chemicals were found to depress in isotonic concentrations but not in one-half that strength we could not conclude that they had no depressor effects in the weaker solution, because the stimulating action of the hypotonicity may counteract the depressor action of the chemical. It is, furthermore, evident that we cannot compare the depressor effects of different concentrations of a depressant chemical by using it in isotonic and in various degrees of hypertonic and hypotonic concentrations, because in the concentrations above the isotonic the depressant action of the chemical is augmented by the depressant action of hypertonicity; in concentrations below the isotonic the specific influence of the chemical is counteracted by the stimulating influence of hypotonicity, while in the isotonic concentration the action of the chemical is influenced by neither factor.

The various theories advanced to account for the mechanism of the stimulating and depressing actions on the living tissues of chemicals that do not serve as food or sources of energy for the tissues will be referred to more extensively in the last section of the paper after the experimental data have been summarized. The work was not pursued with the view of supporting or combating any theory, but rather to establish some necessary preliminary facts. Suppose it is found that when the plasma surrounding the Limulus heart ganglion is replaced by a solution composed of two hundred parts of plasma to one part $\frac{6}{10}$ *n* nitric acid, the ganglionic rhythm is augmented; or suppose that augmentation or depression of the ganglionic rhythm follows the replacing the plasma by a solution composed of four thousand parts of plasma to one part $\frac{6}{10}$ *n* ammonium hydrate or fifty parts of plasma to one part $\frac{6}{10}$ *n* barium chloride, we may conceive of at least three different factors to either of which or to any combination of which this change in the rhythm may be due. 1. The change in the rhythm may be an indirect effect of the chemical and physical changes produced in the plasma by the added chemical, rather than a direct effect of the chemical on the tissue. 2. The added chemical may enter the cells of the tissue and produce the change in the rhythm by acting on some processes or constituents

within the cells themselves. 3. The added chemical may produce the change in the rhythm by a direct action on the cell walls in a way altering the permeability to the substances in solution outside as well as inside the cells. When more than one of these factors are involved — and that is probably always the case — the different factors may act in opposite directions. The relative importance of the different factors is probably different for different tissues, as we know that tissues differ chemically and that different tissues exhibit differences in permeability to the same chemical.

The changes in the equilibrium of a complex solution like the blood produced by any added chemical may be inferred in part from physical chemical data, but the actual influence of such changes on the tissues can only be determined by producing the same changes in the blood by other than chemical means. I have as yet no conclusive data that will enable me to state which mechanism is involved in the stimulating or depressing influence of any chemical worked with. Hence the term specific action or influence of a chemical does not necessarily imply a direct action of the chemical on the tissue in question.

It is not necessary to describe the method of preparing the heart and the devices for graphic registration of the heart rhythm under conditions allowing the action of a solution to be studied on the heart ganglion apart from the heart muscle and *vice versa*. The reader is referred to the paper on the effects of temperature of the Limulus heart for an account of these methods.

The experiments were necessarily performed on excised hearts. The excised Limulus heart will continue in rhythm for a long time (twenty-four to forty-eight hours) when kept in plasma or sea water at not too high temperature (10°–15° C.), but the rhythm becomes gradually more and more feeble, and this is particularly the case with hearts in which the ganglion has been isolated in the posterior region. In prolonged experiments on the influence of certain solutions the results are therefore complicated by the gradual failure of the rhythm that takes place in the plasma or sea water alone. Few of the experiments here summarized extended over two or three hours. In fact, most of them were of much shorter duration. My results deal, therefore, only with the more immediate effects of these chemicals on the ganglion and the muscle. The chemicals used were of Kahlbaum's and Merck's manufacture.

II. The Action of Certain Non-Electrolytes on the Heart Ganglion and the Heart Muscle.

The action of isotonic solutions of sugars, urea, and glycerine on the rhythm of the heart ganglion has already been briefly considered in relation to the question whether the immediate stimulus to the heart rhythm is to be referred to the electrolytes of the blood surrounding the heart tissues or to some substances or conditions within the automatic cells themselves.[1] There only remains to be recorded the fact that these non-electrolytes appear to act on the heart muscle (and nerve endings) solely in the direction of depression, while on the heart ganglion they act primarily as stimulants.

1. **The action of the sugars.** — When the heart ganglion is placed in isotonic solutions of cane sugar, dextrose, lævulose, or lactose, the rhythm is primarily augmented both in rate and in intensity. The augmentation may be accompanied by irregularity in the rhythm or the irregularity may first appear at the stage where the augmentation is followed by depression. The depression of the rhythm is gradual, till after a fifty to ninety minutes bath in the sugar solution the rate of the nervous discharges from the ganglion is much slower than prior to the sugar bath, and the intensity of the nervous discharges too feeble to produce contractions in the muscle. Only vigorous ganglia maintain their rhythm for that length of time in a pure sugar solution. Feeble ganglia, that is, ganglia exhibiting for some reason a weak and irregular rhythm, are brought to a standstill in a pure sugar solution within ten to twenty minutes. There appears to be no difference in the quality and intensity of action of the different sugars employed. The amount of primary stimulation of the ganglion by the isotonic sugar solutions varies from one preparation to another. In two or three preparations practically no augmentation of the intensity of the nervous discharges was produced, while the rate of the rhythm was distinctly increased. There is, hence, no escape from the conclusion that *in isotonic solutions these sugars have a primary stimulating action on the heart ganglion.* What is the mechanism of this stimulating action? The temperature factor is eliminated, as the sugar solutions were of the same temperature as the plasma or sea water which they replaced. Impurities in the sugars can also be ruled out, particularly in the case of the cane sugar. The possibility that the stimulating action might be due to a slight

[1] Carlson: This journal, 1906, xvi, p. 221.

degree of hypotonicity of the sugar solutions, as compared to the plasma, is ruled out by the following experiment. I have shown in the paper referred to above that hypertonicity depresses while hypotonicity stimulates the ganglion. Now, as a matter of fact, a molecular sugar solution is slightly hypertonic to the Limulus ganglion, and should by virtue of this hypertonicity depress the ganglionic rhythm primarily. But the depressor influence due to the slight degree of hypertonicity is more than counterbalanced by some stimulating factor in the sugar solution, because the solution most nearly isotonic (0.95 molecular) as well as the slightly hypertonic solutions produce primary augmentation of the rhythm.

When these isotonic sugar solutions are applied to the muscle in such a manner that the ganglion is not affected, the amplitude of the contraction is diminished without any primary augmentation. — Out of the numerous preparations tested on this particular point only two exhibited a slight increase in the amplitude of the contraction for a minute or two following the application of the sugar solutions to the muscle. I am inclined to ascribe these exceptions to experimental errors, the ganglion being in some way stimulated, or the temperature of the sugar solutions being slightly different from the plasma replaced.

The rate of the contractions cannot be influenced by solutions applied to the heart muscle (and nerve endings) alone, as the rate is determined by the ganglion. The only effects such solutions can produce are an increase or a decrease in the amplitude of the beats, due to some action on the muscles or on the motor nerves and nerve endings.

The pure sugar solutions finally render the muscle unable to respond to nervous discharges from the ganglion or the nervous impulses are prevented from reaching the ganglion by a depressor action on the nerves and nerve endings. In isotonic sugar solutions the heart muscle usually ceased to contract sooner than the complete cessation of the ganglionic rhythm, that is, in case of ganglia in good condition. But the comparative measurements were not of sufficient number to state this difference numerically, because of the great individual variations.

The tracings in Fig. 1 show a typical record of the depressant action of the isotonic sugar solutions on the heart muscle. The lower record is from the posterior end of the heart, showing that the depression of the rhythm of the anterior end bathed in the sugar is not due to a change in the activity of the ganglion.

2. **The action of urea.** — Isotonic solutions of urea act on the heart ganglion in a way similar to the isotonic sugar solutions, only the action of the urea solution is much more rapid and intense. This applies both to the primary stimulating action and to the subsequent depression and final cessation of the rhythm. While a vigorous ganglion may continue in activity for fifty to ninety minutes in the

FIGURE 1. — One-half the original size. Simultaneous tracings from the anterior end (upper record) and the posterior end (lower record) of the Limulus heart. Nerve cord extirpated anteriorly, the two ends of the heart being connected by means of the lateral nerves only. *X*, the plasma surrounding the anterior end replaced by $\frac{1}{20}$ molecular cane sugar solution. *X'*, the sugar solution replaced by plasma, showing depression of the heart muscle by the pure sugar solution, not preceded by augmentation. The rhythm of the ganglion continues unaffected.

sugar, it is brought to a complete standstill within three to five minutes in the urea solution. The urea solution always produces irregularity of the rhythm simultaneously with the augmentation. The rhythm reappears after replacing the urea by plasma or sea water, the returning rhythm being very rapid and irregular as a rule. As this is not characteristic of the rhythm after a standstill in a sugar bath, it would seem that the urea has a greater injurious action on the ganglion than has the sugar.

The primary stimulating action of the urea solution on the ganglion is so marked and constant that it can hardly be due to any other factor than the urea itself; but the same precautions were taken as in the case of the sugar solution to exclude possible impurities in the urea, differences in temperature, lack of isotonicity, etc. The primary stimulating action of urea on the heart ganglion is further shown by the fact that a solution of urea of 0.6 molecular strength in plasma or sea water acts as a primary stimulant. This solution is, of course, greatly hypertonic, and should therefore depress the ganglion, but the depressor influence due to the hypertonicity is more than counterbalanced by the stimulating action of the urea.

The action of isotonic solutions of urea on the heart muscle apart

from the ganglion is the same as that of the isotonic sugar solutions, only much more intense and rapid.

3. **The action of glycerine.** — The influence of isotonic solutions of glycerine on the heart ganglion is similar to that of the sugar and the urea solutions. Both in its primary stimulating and its subsequent depressor action the glycerine acts more intensely and rapidly than the sugars, but less so than the urea. The depression of the rhythm following the primary stimulating action by the glycerine is always accompanied by irregularities in the rate and the intensity of the nervous discharges.

The action of glycerine in isotonic solutions on the heart muscle is the same as that of the sugar solutions, that is, a depressor action not preceded by stimulation.

4. **On the mechanism of stimulation.** — The sugars and the glycerine may be oxidized by the active ganglion cells. Their stimulating action on the ganglion may then be due to energy furnished the cells. This does not account for the opposite action of the sugars on the heart muscle, nor can the explanation be extended to the urea. That the isotonic sugar solution acts as a primary stimulant to the ganglion because of oxidation of the sugar by the ganglion is improbable. The fact is, that the sea water is practically as efficient a medium for the ganglion as the plasma, although the latter contains sugar, while the former does not. The sugar contents of the medium may be greatly increased, isotonicity being at the same time maintained, without augmenting the rhythm.

Hypotonicity stimulates the ganglion. Some of the changes following the immersion of the ganglion in a diluted plasma or sea water are necessarily the same as those following the immersion of the ganglion in an isotonic solution of a non-electrolyte like sugar or urea. In both cases there will be, firstly, an outward diffusion of the electrolytes of the blood in the intercellular spaces and hence a dilution of the salts in solution about the cells; secondly, in all probability a diffusion of some of the constituents normally held within the cells out into the liquid of the intercellular spaces. But while these factors are the same in the hypotonic solution and the isotonic solution of a sugar, it does not follow that these are the factors determining the stimulating action. This caution appears all the more cogent as the hypotonic solution and the isotonic solution of a sugar and glycerine have opposite actions on the heart muscle.

Some of the salts in the blood stimulate the ganglion (K, Na)

while others depress the ganglion (Ca, Mg). When the ganglion is immersed in an isotonic solution of a non-electrolyte the salt in the blood contained within the intercellular spaces will gradually diffuse into the solution. If the depressor salts (Ca, Mg) diffused into the solution considerably faster than the stimulating salts the primary stimulating action of a solution of sugar or urea might then be due simply to the temporary condition of preponderance of the stimulating salts about the cells. This explanation might be tested by determining the rate of diffusion of these salts in molecular solutions of sugar, urea, and glycerine. I do not know of any such determinations. But it seems highly improbable to me that the rate of diffusion of the chlorides and sulphates of calcium and magnesium should be as much greater into a molecular urea solution than into a molecular sugar solution as the greater stimulating action of the urea solution demands on this theory.

It is claimed by some investigators that a small amount of sugar in an artificial circulating medium is beneficial to the heart rhythm in vertebrates. This may, however, be explained in other ways than by a direct stimulating action of the sugar on the heart tissues. I do not know of any recorded observations to the effect that the ultimate depression and cessation of the rhythm of the vertebrate heart in an isotonic solution of pure sugar is preceded by an augmentation of the rhythm similar to that of the Limulus heart ganglion. As the sugar solution may have primarily an opposite action on the heart ganglion and the heart muscle in vertebrates similar to that on the Limulus heart, the results in the vertebrate heart are less readily interpreted.

Intravenous injection of glycerine accelerates the mammalian heart in case the vagi are severed and produces convulsions. The convulsions are probably due to the stimulating action of the glycerine on the central nervous system.

As far as I know urea does not have any marked effect on the mammalian heart when increased above the normal amount in the blood.

III. The Specific Influence of the Inorganic Salts of the Blood on the Heart Ganglion and the Heart Muscle.

With the exception of the copper of the hæmocyanin and a trace of silicates the inorganic constituents of the Limulus blood are the same as those of the blood of vertebrates, the difference being only

in absolute and relative percentage of the different constituents.[1] The electrolytes of the Limulus blood are therefore the chlorides, carbonates, sulphates, and phosphates of sodium, potassium, calcium, magnesium, and possibly ammonium.

1. **The specific influence of the blood salts on the heart ganglion.** a. *The action of the sodium salts.* — When the isolated ganglion is transferred from plasma or sea water to an isotonic solution of pure sodium chloride the ganglionic rhythm is primarily augmented both in the rate and in the intensity of the nervous discharges. This stimulating action of the pure sodium chloride on the ganglion appears within less than half a minute after immersion of the ganglion in the solution. The augmented rhythm is usually perfectly regular. The stimulation is followed by depression of the intensity of the nervous discharges, irregularity, and ultimate standstill or cessation of the rhythm. The relative length of time a ganglion continues in activity in a pure sodium chloride solution isotonic with the blood was not determined accurately. Ganglia in poor condition are brought to a standstill sooner than ganglia in good condition. The rhythm may be restored by removing the ganglion to plasma or sea water, provided it has not been kept too long in the sodium chloride after cessation of the rhythm.

The isotonic solution of pure sodium chloride not only augments the rhythm of an active ganglion, but it may start a rhythm in a quiescent ganglion. For example, a ganglion kept in plasma or sea water at a temperature of 8° to 10° C. for two to three days will often be found perfectly quiescent at the end of this time. When such a ganglion is immersed in an isotonic sodium chloride solution of the same temperature as the ganglion itself, the ganglion is usually resuscitated and may continue in activity from twenty to sixty minutes. This inauguration of the rhythm in a quiescent ganglion must be associated with the sodium chloride itself, because aërating or renewing the plasma about the ganglion does not produce these results.

It is not necessary to use the sodium chloride in isotonic concentrations to obtain this stimulating action on the ganglion. The same results are obtained by increasing the relative percentage of the sodium chloride in the blood, the isotonicity of the medium being at the same time maintained. Thus a ganglion removed from the plasma to a solution made up of plasma and $\frac{6}{10}$ *n* sodium chloride in the proportion 1–1 invariably exhibits an augmented rhythm, but the

[1] Analysis by Mr. H. McGuigan, the results soon to be reported.

augmentation is not so great as in the pure sodium chloride. When the proportion of sodium chloride in the mixture is less, the stimulating action on the ganglion is correspondingly diminished.

By the same method of experimentation the sulphates and carbonates of sodium were also found to have a primary stimulating action on the ganglion. The relative stimulating power to these three sodium salts was not accurately determined, but the chloride seems to act more strongly than the sulphate or the carbonate. The specific influence of the phosphate of sodium on the ganglion was not determined.

b. *The action of the potassium salts.* — The attention was directed mainly to the chloride, in the first place because the influence of this salt on the heart ganglion is of a nature opposite to that on the heart muscle. My interest in the action of potassium chloride on the heart ganglion of Limulus was further increased by the theories of Bottazzi and Howell associating this salt with physiological inhibition of the heart.[1] I have shown in a previous paper that in Limulus the inhibitory heart nerves stop the heart, not by acting on the heart muscle but by inhibiting the automatic heart ganglion.[2] If potassium chloride is in some way associated with physiological inhibition of the Limulus heart, that fact ought to be indicated by its action on the heart ganglion.

Potassium chloride in strengths that affect the heart ganglion at all acts as a primary stimulant to the rhythm. When the isolated ganglion is placed in an isotonic solution of pure potassium chloride the activity of the ganglion ceases within ten to twenty seconds or sooner, but this depressant action is always preceded by augmentation of the rhythm (Fig. 2, *D*). In isotonic concentrations of this salt the stimulating action on the ganglion appears practically instantaneously and leads to a tonus and incomplete tetanus contraction of the heart muscle for a few seconds or till the ganglionic rhythm ceases entirely. A ganglion that exhibits a slow and feeble rhythm ceases its activity in an isotonic solution of potassium chloride sooner than does a vigorous ganglion. In fact, a ganglion that acts very slowly and feebly may be arrested by the $\frac{6}{10}$ *n* potassium chloride after one or two augmented nervous discharges. Such a slight indication of a primary stimulating action might easily pass unnoticed in case of

[1] For the literature see HOWELL: This journal, 1906, xv, p. 280.

[2] CARLSON: This journal, 1905, xiii, p. 217.

direct observation, and the inference made that in some cases the potassium chloride has a primary depressant action on the ganglion.

The influence of potassium chloride on the ganglion is best studied in solutions of less than isotonic concentrations. The primary stimulating action appears in concentrations represented by twenty-nine parts of plasma to one part $\frac{6}{10}\,n$ potassium chloride. In concentrations repre-

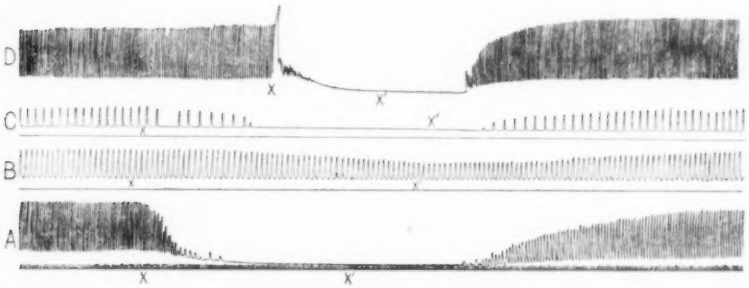

FIGURE 2. — One-half the original size. Tracings from the anterior end of the Limulus heart. Heart ganglion isolated posteriorly and subjected to the solutions. The anterior, or recording part of the heart is immersed in plasma. *A*, x = application of solution of equal parts $CaCl_2$ ($\frac{6}{10}\,n$) and sea water to ganglion. X' = this solution replaced by sea water. Showing depression of the ganglion by the $CaCl_2$. *B*, x = application of an isotonic solution of $MgCl_2$ to ganglion. X' = magnesium solution replaced by sea water. Showing depression of the ganglion. *C*, x = ganglion immersed in an isotonic solution of $CaCl_2$. X' = ganglion bathed in plasma. Showing depression of the ganglion. *D*, x = ganglion bathed in a solution of KCl ($\frac{6}{10}\,n$) and sea water (1–1). X' = potassium solution replaced by plasma. Showing primary stimulation of the ganglion.

sented by nine parts of plasma or sea water to one part $\frac{6}{10}\,n$ of the salt the regular rhythm of the primary augmentation is quickly supplanted by the peculiar irregular rhythm illustrated in Fig. 3. The beats fall into more or less definite groups separated by pauses, the rate of succession of the group beats approaching incomplete tetanus in some cases. This rhythm suggests a possible double action of the salt on the ganglion cells, a stimulating action on one series of physiological processes and a depressor action on another. Even in dilution of the salt represented by plasma and $\frac{6}{10}\,n$ potassium chloride in the proportion of 9–1 the ganglion is quickly brought to a standstill, which is a further indication of the depressant action of this salt, because the augmentation preceding the standstill is not great enough to cause a standstill of the ganglion from exhaustion.

This is further indicated by the fact that the ganglionic rhythm is quickly restored by plasma or sea water.

Potassium sulphate has a primary stimulating action on the ganglion. The influence of the carbonate and the phosphate of potassium was not determined.

c. *The action of the ammonium salts.* — The ammonium salts studied were the chloride, sulphate, and hydrate. *The ammonium chloride has a distinct stimulating action on the heart ganglion* (Fig. 5, *A*). This stimulating action is evident in dilutions represented by fifteen parts of sea water or plasma to one part $\frac{6}{10}$ *n* of the salt. The greater the concentration of the salt the greater the stimulation of the ganglion. The stimulation is manifested both in the rate and the intensity of the nervous discharge from the ganglion. No indication of the peculiar double action described in connection with the influence of the potassium chloride on the ganglion was obtained from the ammonium chloride. On long continued action and in concentrations greater than four parts of sea water to one part $\frac{6}{10}$ *n* of ammonium chloride the primary stimulation is followed by irregularity and final depression. The action of the ammonium sulphate was not studied extensively, but sufficiently to show that the influence of the sulphate is the same as that of the chloride only less intense. The action of the ammonium hydrate will be considered in another connection.

d. *The action of the calcium salts. — Calcium chloride in concentrations up to* $\frac{6}{10}$ *n depresses the ganglion without any indication of a primary stimulation* (Fig. 2, *A*). The depressor effects are manifested both in the rate and in the intensity of the nervous discharges. The diminution of the strength of the nervous discharges is on the whole more marked than the slowing of the rate, and with weak concentrations of the salt it is possible to obtain a slight diminution in the height of the contractions without any appreciable slowing of the rate. In isotonic concentration the calcium chloride stops the ganglionic rhythm sometimes within a minute. This cessation of the ganglionic activity is accompanied by tonus relaxation of the heart muscle, as the normal tonus of the heart muscle is dependent on the physiological activity of the ganglion. On returning the ganglion to plasma or sea water the rhythm returns gradually.

Increasing the concentration of calcium chloride in the plasma to the amount represented by twenty-nine parts of plasma to one part $\frac{6}{10}$ *n* of the salt rarely produces any appreciable inhibition of the ganglion but concentrations represented by the proportions 19–1, 9–1, and 4–1

produce distinct inhibition. The depressor effects of the salt on the ganglion is therefore directly proportional to the concentration. Concentrations greater than $\frac{6}{10}$ *n* cannot be worked with because they introduce the depressing influence of hypertonicity.

The carbonate, sulphate, and phosphate of calcium depress the ganglion. Only a few experiments were made using the carbonate and the phosphate.

e. *The action of the magnesium salts.* — Only the chloride and the sulphate were used in these experiments. *These two salts depress the ganglionic rhythm without primary stimulation*, the depressant action of the sulphate being less than that of the chloride and the depressant action of either salt being on the whole less than the corresponding salts of calcium. In isotonic concentrations the magnesium chloride will completely inhibit the ganglion within five to ten minutes after immersion, vigorous ganglia maintaining the rhythm for a longer period than feeble ganglia. The depression appears both in the rate and the intensity of the nervous discharges; weaker concentrations depressing mainly the intensity. On returning the ganglion to plasma or sea water the rhythm gradually returns (Fig. 2, *B*).

Summary of the specific influence of the organic salts of the blood on the ganglionic rhythm: 1. Isotonic solutions of either of the inorganic salts of the blood bring the ganglion to a standstill without killing the tissue, that is, after such a standstill the rhythm of the ganglion may be restored by plasma or sea water. Either of the inorganic salts of the blood may therefore be said to act as a "poison" to the ganglion when the ganglion is bathed in a pure solu-

FIGURE 3. — About two-thirds the original size. Tracings from the anterior end of the Limulus heart. Ganglion isolated posteriorly and subjected to the action of an increased concentration of KCl. *X* = application of a solution of 9 parts sea water + 1 part KCl (6.9 %) to ganglion. *X'* = this solution replaced by plasma, showing primary stimulation and typical grouping of the nervous discharges from the ganglion.

tion of the salt. 2. The sodium potassium and ammonium salts in concentrations above that of the blood up to $\frac{6}{10}$ *n* augment the ganglionic rhythm prior to the standstill, the potassium salts being the more powerful stimulants and at the same time the more injurious to the ganglion. In similar concentrations the salts of calcium and magnesium depress the ganglionic rhythm without primary stimulation.

2. **The specific influences of the salts of the blood on the heart muscle.** — The specific influence of the inorganic constituents of the blood on the heart muscle (and nerve endings) apart from that on the heart ganglion, may in the Limulus heart be studied by two different methods. The ganglion may be entirely extirpated and the heart, thus rendered quiescent, immersed in the different solutions, and the variations in the excitability and contractility of the heart muscle tested by artificial stimulation. Or the ganglion may be removed from the anterior end of the heart only, and the heart transected in the third segment, having the lateral nerves intact. The anterior end of the heart is now free from ganglion cells, but continues to beat in synchrony with the posterior end of the heart in consequence of the impulses reaching it through the lateral nerves. The ganglion is kept under normal and constant conditions, the solutions of the various salts applied to the ganglion free anterior end and the variations in the amplitude of the contractions of this end recorded. In these experiments I have followed the latter method almost exclusively because that enables us to determine the influence of the salts on the response of the muscle to the normal or physiological stimulus. Neither method enables us to determine whether the specific effects of a solution is due to a direct action on the heart muscle or the action on the motor nerve ending in the muscle. Provisionally, I speak of the effects as due to a direct action on the heart muscle.

a. *The action of the sodium salts.* — Isotonic solutions of sodium chloride or sodium sulphate produce a series of rhythmical contractions in the heart muscle after it has been completely severed from the ganglion.[1] The latent period between the immersion of the quiescent heart muscle in solutions of the sodium salt and the beginning of the rhythm is from thirty to forty minutes. In plasma or sea water the heart muscle remains permanently quiescent, in this respect resembling the ventricle of the frog or the tortoise after separation from the auricles.

[1] CARLSON: This journal, 1905, xii, p. 491.

When an isotonic sodium chloride solution is applied to the anterior end of the heart from which the ganglion has been removed in that region, the lateral nerves being left intact, the strength of the contractions is usually increased gradually for a short period. This stimulating action may not be very marked. It is followed by depression of the amplitude of the contractions. The final depressor action of the pure sodium chloride on the muscle in some cases brought the anterior end of the heart to a standstill before the appearance of the rhythm due to the direct action of the salt on the heart muscle. If we are right in ascribing this depressor effect to the action of the sodium chloride on the muscle itself, it follows that the idiomuscular rhythm of the heart muscle in pure sodium chloride may appear after the heart muscle has been so greatly altered that it can no longer respond to the nervous impulses from the ganglion. It is, of course, possible that the standstill of the anterior end of the heart preparation in pure sodium chloride prior to the appearance of the idiomuscular rhythm is due to a depressor action of the salt on the motor nerve endings rather than on the muscle itself. This point is being investigated further in the hope of throwing some light on the relation between the rhythm of automatic tissues and the rhythm of non-automatic tissues such as skeletal muscle and frog's or tortoise's ventricular muscle in a pure sodium chloride solution. It has appeared to me that many of the conclusions regarding the nature and mechanism of the rhythm of automatic tissues based on studies of artificial rhythms in tissues not automatic under normal conditions are not cogent. The artificial character of the sodium chloride rhythm in ventricular tissue and skeletal muscle is strikingly illustrated by Guenther's observations that this rhythm is confined to the parts of the muscle in immediate contact with the pure sodium chloride solution, and spreads to more remote parts as the salt diffuses through the tissues.[1] The influence of the carbonate and phosphate of sodium on the heart muscle was not determined.

b. *The action of the potassium salts.* — The only potassium salt worked with on the heart muscle was the chloride. *When the ganglion free heart muscle is bathed in isotonic potassium chloride the strength of the contractions is diminished without primary augmentation* (Fig. 4). The beats are completely abolished usually within less than a minute, and the contractions are restored by bathing that part of the heart in plasma or sea water. Concentrations of

[1] GUENTHER: This journal, xiv, p. 72.

the salt represented by plasma and $\frac{6}{10}$ *n* potassium chloride in the proportions 1–1, 4–1, 9–1 act in the same way, only less rapidly and intensely than the pure isotonic solution. The depressant action of the salt is thus proportional to the concentration. In two instances I obtained a slight primary increase in the strength of the contraction when the muscle was bathed in the weaker (9–1) concentrations of the salt, but this might have been due to some other factor. No primary stimulating action on the heart muscle was ever observed

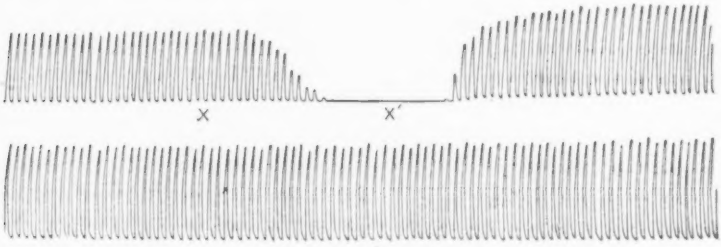

FIGURE 4. — Two-thirds the original size. Simultaneous tracings from the anterior end (upper record) and posterior end (lower record) of the Limulus heart. The two ends of the heart connected by the lateral nerves only. Ganglion extirpated in anterior end. *X* = application of sea water + KCl ($\frac{6}{10}$ *n*), 4–1, to the anterior end. *X'* = this solution replaced by sea water, showing depression of the muscle without primary stimulation.

in the stronger concentrations of the salt. That the action is on the muscular end and not a reflex through the ganglion on the posterior end of the heart is shown by taking simultaneous tracings from the two ends of the heart. It is, therefore, evident that the potassium chloride (and possibly also the sulphates, carbonates, and phosphates) act on the heart muscle in a way opposite to that on the heart ganglion.

c. *The action of the calcium and the magnesium salts.* — The chlorides and sulphates of these two metals were tried. *The action of these salts on the heart muscle is the same as on the heart ganglion.* In pure isotonic solutions or in various rations of mixtures of the $\frac{6}{10}$ *n* solution with plasma the amplitude of the contractions is diminished without primary augmentation. These salts have therefore no primary stimulating action on the heart muscle either in the direction of contractility or excitability.

d. *The action of the ammonium salts.* — *Ammonium chloride depresses the heart muscle without primary stimulation.* The action of

chlorides of potassium and ammonium on the heart muscle is therefore identical, the difference being only in degree of intensity, the potassium salt being by far the more powerful depressant. This is illustrated in tracings *B*, Fig. 5, and the upper tracing in Fig. 4, the latter tracing showing the depressant action of the potassium chloride and the former that of the ammonium chloride. The concentration of the two salts is the same, or four parts sea water to one part $\frac{6}{10}$ *n* of the respective salt.

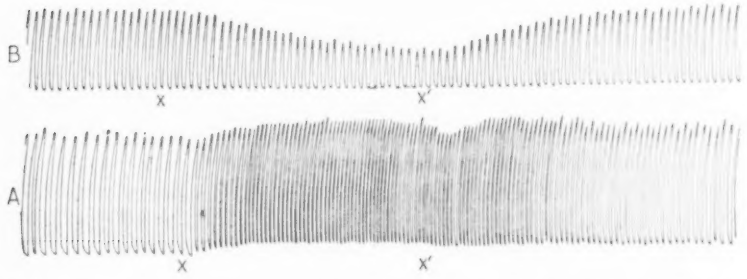

FIGURE 5. — About five-ninths the original size. Tracings from the anterior end of the Limulus heart. *B*, ganglion isolated posteriorly, anterior or recording end immersed in plasma. *X* = ganglion bathed in mixture of four parts sea water to one part NH_4Cl ($\frac{6}{10}$ *n*). *X'* = this solution replaced by sea water, showing primary stimulation of ganglion. *B*, ganglion extirpated in anterior end, the two ends of the heart connected only by the lateral nerves. *X* = anterior end bathed in mixture of four parts sea water to one part NH_4Cl ($\frac{6}{10}$ *n*). *X'* = this solution replaced by sea water, showing primary depression of the heart muscle.

In ammonium chloride we have, therefore, another chemical that produces qualitatively different effects on the two tissues of the Limulus heart.

Summary of the action of the blood salts on the heart muscle. — 1. The salts of calcium, magnesium, potassium, and ammonium depress the heart muscle without primary stimulation. The sodium salts produce primarily a slight augmentation in the strength of the contractions. 2. The salts of sodium, calcium, and magnesium have primarily the same influence on the heart muscle and the heart ganglion, while the potassium and the ammonium salts produce qualitatively different effects on the two heart tissues.

3. **The chemical conditions of automatism of the Limulus heart compared with those of the vertebrate heart.** — The literature on the specific influence of the different salts of the blood on the rhythm of

the vertebrate heart is very extensive. There is some disagreement between the different investigators as to the actual or observable facts, not to mention the disagreements in the interpretation of these facts. Much work has been done on the specific influence of the different blood salts in starting, maintaining, or inhibiting rhythmical contractions in strips from the ventricular apex of the frog and the tortoise (Howell, Greene, Lingle, and others). But as not even the whole ventricle severed from the auricles of the tortoise and the frog is automatic under the normal conditions, that is, when bathed in serum or in an isotonic solution of the serum ash, contractions of isolated parts of the ventricle is in every case an artificial rhythm, and the influence of the salts on this artificial rhythm is not necessarily the same as that on the normal rhythm or on the parts of the heart automatic under normal conditions. The interpretation of much of the work done on the influence of the salts on the normal rhythm is rendered difficult by the fact that all observers have not in every case worked with isotonic solutions. The specific influence of a salt is thus complicated by hypertonicity or hypotonicity. The blood constituents worked with are mainly the chlorides of sodium, potassium, and calcium. So far as I can learn from the literature, the specific influence of the magnesium salts of the blood appears not to have been studied at all, although these are as constant and presumably as important factors in maintaining the necessary conditions for the heart rhythm as the salts of sodium, potassium, and calcium.

It is true both for the Limulus heart ganglion and the vertebrate heart that of all the salts of the blood acting singly and in isotonic concentrations sodium chloride is the least injurious; that is, when the beating heart is bathed in an isotonic solution in either of the salts, the sodium chloride maintains the activity the longest. In the frog, replacing the blood with an $\frac{n}{8}$ sodium chloride solution usually produces an augmentation of the rate, while the beats become gradually weaker. The augmentation of the rate may provisionally be ascribed to a primary stimulating action of the sodium chloride. The specific influence of sodium chloride is therefore the same on the Limulus and the vertebrate heart, or a primary stimulation, followed by depression and final cessation of the rhythm, while the tissues still remain in such condition that the rhythm reappears on placing the hearts in serum, or Ringer's or Locke's solution. There is still further similarity between the action of sodium chloride on Limulus heart muscle and on the tortoise and frog's ventricle. Lingle has

shown, and it has been confirmed by Howell and his students, that a pure sodium chloride solution causes rhythmical contraction in ventricular strips, and that the salt must be present in any solution that will start a ventricular strip in normal condition beating. The same is true for the Limulus heart muscle, or parts of the heart from which the ganglion has been removed.

The two most recent investigators disagree as to the primary influence on mammalian heart rhythm of a slight increase of the potassium chloride of the blood. According to Braun a slight increase of the potassium chloride acts as a stimulus to the rhythm of the isolated heart, while greater concentration depresses the rhythm.[1] Sollmann makes a similar statement regarding the action of the potassium ion on the heart.[2] According to Hald the potassium chloride content may be increased to twice that of the normal blood, 0.07 to 0.08 per cent, without producing any marked effect on the heart rhythm, either in the mammal or the frog, but greater concentration depresses the rate, and ultimately the strength of the beats.[3] In the intact mammal (rabbit) the inhibitory effect of the potassium chloride on the heart is sometimes preceded by augmentation. Hald interprets the primary stimulating action as due to depressor effects of the salts on the vagus centre in the medulla. In the case of the frog's heart Hald found that the influence of an increase in the potassium chloride of the blood depends to some extent on whether the solution is circulated through the heart or the heart immersed in the solution. Bottazzi describes only depressor, or inhibitory effects of chloride, nitrate, and carbonate of potassium on the frog's heart. That is also the conclusion of Howell[4] with regard to the specific influence of potassium salts on the heart.

It has already been noted that according to Braun a slight increase in the potassium chloride content acts as a stimulant to the heart. Hald cites two other investigators (Nobiling, Stockvis) to the same effect. Gross claims that potassium chloride produces an increased excitability of the vagi of mammals (rabbit) prior to the depression and loss of excitability.[5] The slowing of the heart by potassium

[1] Braun: Archiv für die gesammte Physiologie, 1904, ciii, p. 476.

[2] Sollmann: Text-book of pharmacology, 1901, p. 553.

[3] Hald: Archiv für experimentelle Pathologie und Pharmakologie, 1905, liii, p. 227.

[4] Howell: *Loc. cit.*

[5] Gross: Archiv für die gesammte Physiologie, 1903, xc, p. 264.

chloride has also been interpreted as due to stimulation of the inhibitory nervous mechanism in the heart rather than to a direct depressor action on the automatic tissue.

On the neurogenic theory of the heart beat the slowing of the rhythm must be due to an inhibitory action on the automatic ganglion or ganglia, while a diminution in the amplitude of the beats may be due to a depressor action on the ganglion and the nerves, or on the muscle. Now it is a fact that most of the investigators claim that potassium chloride diminishes the rate of the vertebrate heart without primary stimulation, and that this diminution in rate is usually accompanied by augmented strength of the beats, while greater concentration depress both the rate and the strength of the contractions. Granted that this is correct,—that potassium chloride has only a depressor influence on the vertebrate heart,—how are we to reconcile the apparent primary stimulating action of the same salts on the heart ganglion of Limulus? We have seen that the potassium chloride depresses the Limulus heart muscle without primary stimulation. Now, if the salt depressed the strength of the beats of the vertebrate heart primarily, without affecting the rate, this might have been brought into agreement with the results on the Limulus heart by assuming that the vertebrate heart muscle is more readily affected by the salt than is the heart ganglion, so that the effect on the former obscures the influence on the latter. The primary stimulating action of the salt on the Limulus heart ganglion appears in the strength, and not in the rapidity of the nervous discharges. In fact, a slight indication of a primary slowing (Fig. 3, *B*) may sometimes be made out from the tracings. The apparent augmentation appears when the peculiar double or grouped rhythm sets in, that is, in the diminutive beats in the diastolic phase of the abnormally slow and vigorous beats. Those very slow and vigorous nervous discharges apparently represent the original fundamental rhythm of the ganglion, in which case the fundamental ganglionic heart rhythm of Limulus is primarily slowed in rate and strengthened in intensity, just as seems to be the case in the vertebrate heart. The typical digitalis effect of potassium chloride on the vertebrate heart, that is, the great increase in the strength of the beats accompanying the slowing of the rate, cannot be accounted for by the mere slowing, because no such great difference in the contraction strength is obtained by changing the rate of artificial contractions of the isolated ventricle from 80 to 40 per minute by changing the rate of stimulation. To infer from the

results on the Limulus heart ganglion the increased amplitude of the beats in the vertebrate heart is due in part to increased intensity of the nervous discharges from the automatic ganglion or ganglia. With greater concentration of the salt or on long continued action of a weak concentration, the beats are weakened by the direct depressor action of the salt on the heart muscle as well as by the gradual weakening of the nervous discharges from the automatic ganglion following the period of primary stimulation.

The possibility also remains that the primary slowing is due to stimulation of inhibitory nervous mechanism in the heart.

The primary stimulating action of the potassium chloride on the rest of the vascular system in mammals as shown by the initial vaso-constriction following the increase of the salt in the blood appears to be well established. The experiments of Rothenberg (cited from Hald) and Hald[1] seem to prove that this is a peripheral effect, that is, due to a stimulating action on the nervous tissues or the muscular tissues in the walls of the blood vessels.

There is very little literature on the influence of ammonium salts on the vertebrate heart. I have not seen any reference to the action of the ammonium salts on the heart of cold-blooded vertebrates. Intravenous injection of ammonium chloride or nitrate depresses the heart rhythm by stimulation of the vagus centre in the medulla. If the experiments are performed after double vagotomy the heart rhythm is primarily augmented. This may be due in part to the increased blood pressure in consequence of vaso-constriction. Cushney concludes that the direct action of the ammonium salts on the heart must be considered among the unsettled questions.[2] Formánek came to the conclusion that ammonium chloride in weak concentrations acts as a primary stimulant to the mammalian heart.[3] The stimulating action of the ammonium salts on the central nervous system of mammals is a well-established fact.

The little work that has been done on the influence of the ammonium salts on the vertebrate heart seems to point to the conclusion that its action on the heart is the same as on the central nervous system. In weak concentration they act as primary stimulants; in greater concentrations the primary stimulating action is rapidly followed by depression. If this is a correct interpretation of the literature it is

[1] HALD: *Loc. cit.*

[2] CUSHNEY: Text-book of pharmacology, 1905, p. 497.

[3] FORMÁNEK: Archives de pharmakodynamie, 1900, vii, p. 229.

obvious that action of the ammonium salts on the Limulus heart is the same as that on the vertebrate heart. The stimulating phase of the action is brought about by the stimulating action on the heart ganglion of concentrations of the salts too weak to produce their depressive effects on the heart muscle. The depressor phase is due to the depression of the automatic ganglion following the primary stimulation as well as to the direct depressor action on the heart muscle.

As regards the specific influence of the calcium salts there appears to be a fundamental difference between the Limulus and the vertebrate heart. We have seen that the chloride of calcium in strengths that appreciably influence the rhythm up to isotonic concentration depresses both the heart ganglion and the muscle and nerve part of the heart, while in the case of the vertebrates it appears to be well established that an increase of the calcium content of the blood augments the strength of the heart beats and thus acts as a stimulant. This seems to be true both for cold-blooded and warm-blooded vertebrates. In the frog or the tortoise an increase in the calcium content does not accelerate the rhythm; in the mammal the heart rhythm may be accelerated, but this may be a secondary effect or due to the increased blood pressure in consequence of the stronger beat (Langendorff). According to Greene,[1] an increase in the calcium contents in the tortoise serum will produce rhythmical contraction in strips of the tortoise ventricle that remain quiescent in serum alone. This is denied by Langendorff and Hueck for the frog's ventricular apex.[2] That strips of ventricular muscle will beat longer in a mixture of the chlorides of sodium and calcium than in pure sodium chloride does not prove that the calcium salt has any stimulating action. It simply means that the heart rhythm will continue the longer the nearer the circulating medium approaches the normal.

Langendorff and Hueck worked on both the isolated and intact mammalian (cat) heart, obtaining practically the same results by both methods. In the experiments on the intact heart the apparent stimulating action on the heart might have been due to depression of the vagus centre in the medulla by the calcium chloride, as the vagi are usually tonically active in the cat, and Langendorff and Hueck do not appear to have sectioned the vagi prior to the experiment.

[1] GREENE: This journal, 1899, ii, p. 125.

[2] LANGENDORFF and HUECK: Archiv für die gesammte Physiologie, 1903, xcvi, p. 473.

But this objection does not hold for the experiments on the isolated heart.

Loeb and Lingle explain the favorable effect on the heart rhythm of the addition of a small amount of calcium salts to an isotonic solution of sodium chloride used as irrigating medium by assuming that the calcium counteracts the injurious effects of the sodium.[1] There can be no doubt that either of the blood salts is injurious to the heart tissues in pure solution, but as we do not know the precise mechanism by which these injurious effects are produced it does not follow that the salts have the same effects on the heart tissue when in their normal percentage composition in the blood, and that the normal heart rhythm is the resultant of such toxic and antitoxic or stimulating and depressant actions of the different salt.

The experiments on the isolated heart and the further fact that the heart muscle passes into "contracture" or tonus when the calcium content is raised above a certain limit or when the weaker concentrations are allowed to act on the heart for some time seem to preclude the explanation that the apparent stimulation is due to a depressor action of the calcium salt on some local inhibitory nervous mechanism.

It is well known that an increase of calcium in the blood depresses the central nervous system, apparently without primary stimulation; and thus acts as a narcotic. The chloride of calcium also depresses the excitability of the motor nerves in the frog[2] and inhibits intestinal peristalsis in mammals.[3] It would thus seem that the heart is the only organ or tissue in the vertebrates in which an increase of the calcium content of the blood above the normal acts as a stimulant.

Summary. — 1. The specific influence of the salts of sodium, ammonium, potassium (and magnesium) on the heart activity is similar or the same in Limulus and in the vertebrates. In Limulus the primary action of these salts particularly in weak concentrations is on heart ganglion. In case of salts having a primary stimulating action on the ganglion and a depressant action on the heart muscle the effect on the ganglion is first in evidence. 2. The specific action of the calcium salts on the vertebrate heart appears to be the very opposite to that on the Limulus heart.

4. **On theories of the mechanism of chemical stimulation and depression.** — *a.* Recent workers on the influence of dissociable chemicals

[1] LOEB: Dynamics of living matter, 1906, p. 82.

[2] MATHEWS: This journal, 1904, xi, p. 455.

[3] MACCALLUM: This journal, 1904, x, p. 259.

on living tissues interpret their results in main on the hypothesis of Arrhenius. Chemical stimulation or depression is spoken of as actions of ions. Many of the workers on the relation of the blood salts to heart activity do not refer the specific action of the salts to either ion, while others are inclined to attribute the specific effects to the action of the cathions rather than to the anions. Thus Howell and his students speak of the stimulating action of the calcium ion and the depressing action of the potassium ion on the heart tissues.[1] Loeb and Lingle speak of the cathion in sodium chloride as the stimulating ion. The displacement of certain ions by certain other ions in the hypothetical ion-proteids within the cells has been suggested by some as a possible mechanism of chemical stimulation and depression.[2]

Strong concludes, on theoretical grounds, that chemical and electrical stimulation of nerve is due to the stimulating action of the anions in the axis cylinder.[3] Mathews has carried this idea still further by assuming that all anions stimulate and all cathions depress protoplasmic activity (nerve).[4] This view that cathions depress and anions stimulate living matter is extended by Benedict to the interpretation of the influence of the blood salts on the heart rhythm.[5]

Mathews bases his theory on his extensive studies of the action of chemicals on the motor nerves of the frog. The salient features of the theory are that cathions depress and anions stimulate and that this action is a function of the electrical charges on the ions. The intensity of action of an ion is inversely proportional to its ionic stability or "solution tension"; the lower the solution tension the more powerful the action of the ion on the tissues. The action of any electrolyte on living tissues can therefore be expressed by the ratio between the degree of depressor action of the cathion and stimulating action of the anion. The solution tension of many ions being known it is possible to calculate the relative stimulating or depressing power of many electrolytes. Mathews has made a number of such calculations (Tables XIII, XIV, XV), the theoretical figures showing in many cases a close relationship to his experimental data.

It is difficult to harmonize this theory with the apparent "selective"

[1] HOWELL: This journal, 1902, vi, p. 181.
[2] LOEB: The dynamics of living matter, 1906, pp. 71–98.
[3] STRONG: Journal of physiology, 1900, xxv, p. 427.
[4] MATHEWS: This journal, 1904, xi, p. 455.
[5] BENEDICT: This journal, 1904, xii, p. 192.

action of many chemicals on certain tissues, and the qualitatively different action of the same chemical on different tissues of the same animal. Acids stimulate the gustatory nerve endings, nerve endings in many mucous surfaces, and the nerve endings in the skin of the frog, while they appear to depress nerve trunks and the central nervous system without primary stimulation. Ammonia stimulates the nerve endings of the nasal mucus membranes, while it appears to depress motor nerves without stimulation. Further examples might be enumerated. The probability of some of the assumptions of the theory might moreover be questioned *a priori*. It is not self-evident, for example, why the intensity of the action of the ion should have any relation whatever to the solution tension, even if it be true that the ion acts by giving up its electrical charge to some constituent of the protoplasm. To separate an ion from its charge requires a certain voltage of electricity, and it does not seem unreasonable to assume that it will require an equivalent force to effect the same change of the ion in the living tissue. Unless such a force is present in the tissues the ion will, on the assumption of the theory, not act at all. But we do not wish to follow this line of reasoning any further at present. It is obvious that Mathews's generalizations, if true, greatly simplify the subjects of chemical and electrical stimulation of living matter. The theory should, therefore, be tested on other tissues besides motor nerve trunks, particularly as the latter are never stimulated chemically or electrically under normal conditions of life. With this end in view, the specific action of the following electrolytes was determined on the Limulus heart ganglion and heart muscle.

b. *The action of acids.* — According to Mathews's theory all acids should depress, because of the great depressing power of the hydrogen ion. Theoretically, the depressing power of the hydrogen ion is more than sufficient to overcome the stimulating action of any anion with which it may be associated. In the case of the Limulus heart, however, *the acids have a primary stimulating action on the ganglion and a primary depressant action on the muscle.* Weak concentrations are necessary in order to demonstrate the primary stimulating action of the acids on the heart ganglion, because the activity of the ganglion is quickly stopped by the stronger concentrations. Relatively strong concentrations of the acids may stop the rhythm of a feeble ganglion without any marked primary stimulation. The primary stimulation is best shown in concentrations represented by one part of the isotonic solution of the acid to two hundred or five hundred parts

plasma or sea water. The following acids were tried: hydrochloric, nitric, sulphuric, oxalic, acidic, tartaric, citric, malic, and formic. All these acids acted alike (Fig. 6), differing only in degree of intensity. Hydrochloric acid, for example, stimulates the ganglion more in-

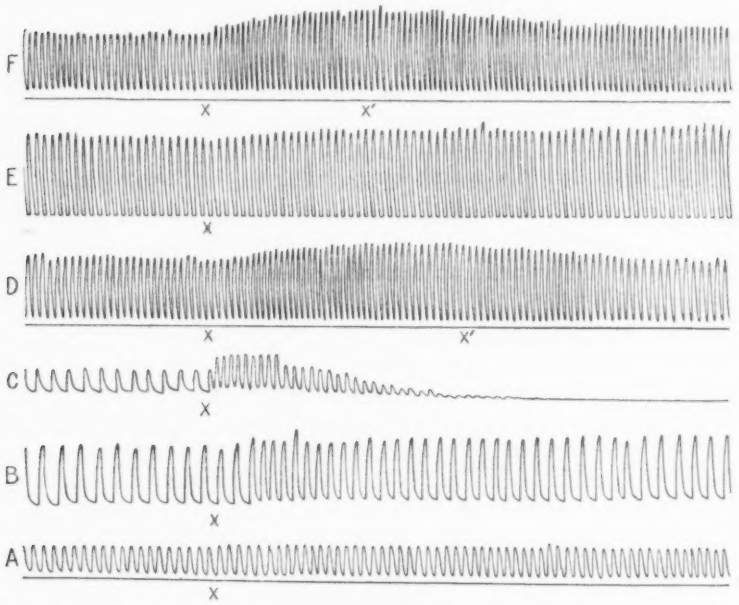

FIGURE 6. — Tracings from the anterior end of the Limulus heart. Ganglion isolated posteriorly and subjected to the chemicals. Recording end of heart immersed in plasma. X = ganglion immersed in the acid solution. X' = acid solution replaced by plasma or sea water. *A*, hydrochloric, 1 part ($\frac{6}{10}$ *n*) to 500 parts sea water. *B*, hydrochloric, 1 part ($\frac{6}{10}$ *n*) to 200 parts sea water. *C*, hydrochloric, 1 part ($\frac{6}{10}$ *n*) to 100 parts sea water. *D*, citric, 1 part ($\frac{6}{10}$ *n*) to 500 parts sea water. *E*, formic, same concentration as in *D*. *F*, tartaric, same concentration as in *D*.

tensely than sulphuric acid. Citric acid is a stronger stimulant to the ganglion than sodium citrate, a fact that is difficult to harmonize with the theory, as the hydrogen ion ought to depress much more intensely than the sodium ion. Solutions of these acids in plasma or sea water of sufficient concentration to barely affect the ganglion have little or no effect on the heart muscle. Strong solutions always depress the strength of the contractions without primary stimulation (Fig. 7). The acids have, therefore, a qualitatively different action on the heart muscle from that on the heart ganglion. The opposite

action of acids on the central nervous system and peripheral nerve endings in vertebrates has already been referred to. Cushney states that in dilute solutions acids augment the activity of the cilia of ciliated epithelium prior to depressing the activity.[1]

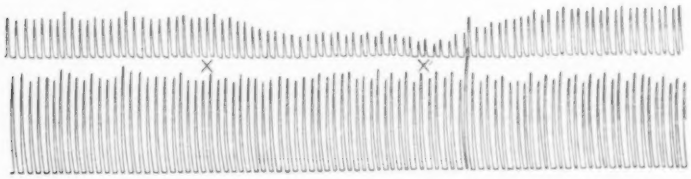

FIGURE 7. — About two-thirds the original size. Simultaneous records from the two ends of the Limulus heart. Ganglion extirpated in anterior end (upper record). X = anterior end immersed in mixture of 200 parts sea water to 1 part HNO_3 ($\frac{6}{10}$ n). X' = this solution replaced by sea water. Showing primary depression of the heart muscle by acids.

c. *The action of the alkalies.* — The hydrates of sodium and potassium stimulate the heart ganglion as well as the heart muscle of Limulus in concentrations represented by one hundred parts of plasma or sea water to one part of an isotonic solution of the alkali (Fig. 8). The stimulating action on the ganglion appears both in the rate and the strength of the nervous discharges. The ammonium hydrate

FIGURE 8. — About two-thirds the original size. Record from anterior end of Limulus heart. Ganglion isolated posteriorly. X = ganglion immersed in mixture of 100 parts of plasma to 1 part KOH ($\frac{6}{10}$ n), showing primary stimulation of the ganglion.

acts very different from the other two alkalies. In the first place, its action is very much more intense. The hydrates of sodium and potassium hardly affect the ganglion or the muscle in dilutions represented by one part ($\frac{6}{10}$ n) of the alkali to five hundred parts of plasma or sea water, while the ammonium hydrate acts strongly on the ganglion in dilutions represented by one part ($\frac{6}{10}$ n) of the alkali to four thousand parts of plasma or sea water. When the ammonium hydrate at this dilution is applied to the ganglion the in-

[1] CUSHNEY: Text-book of pharmacology, 1905, p. 558.

tensity of the nervous discharges is rapidly diminished without primary augmentation, while there is at the same time a slight indication of an acceleration of the rate (Fig. 9, *A*). The acceleration of the rate is too slight to account for the great depression in the intensity of the nervous discharges. The ammonium hydrate, then, appears to have a double and opposite action on the ganglion, viz., a depressor action on the processes involved in the intensity of the nervous impulses, and a slight stimulating action on the processes involved in the rate of the nervous discharges from the ganglion. In

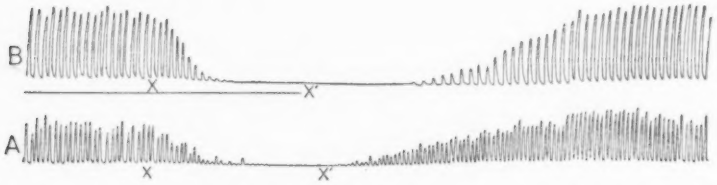

FIGURE 9. — About two-thirds the original size. Tracings from the anterior end of the Limulus heart. *A*, ganglion isolated posteriorly, recording end bathed in plasma. *X* = ganglion immersed in mixture of 4000 parts sea water to 1 part NH_4OH ($\frac{6}{10}$ *n*). *X'* = this solution replaced by sea water, showing primary depression of intensity of nervous discharges with a slight augmentation of the rate. *B*, ganglion extirpated from anterior end. *X* = anterior or recording end bathed in mixture of 1500 parts sea water to 1 part NH_4OH ($\frac{6}{10}$ *n*). *X'* = this solution replaced by sea water, showing primary depression of the heart muscle.

the above dilution (1–4000) the ammonium hydrate does not appear to appreciably affect the heart muscle. In concentrations represented by one part ($\frac{6}{10}$ *n*) of the hydrate to two thousand or fifteen hundred parts of the plasma or sea water, the heart muscle is depressed without primary stimulation (Fig. 9, *B*). The action of the ammonium hydrate on the muscle is therefore opposite to that of the hydrates of sodium and potassium.

The stimulating action of the hydrates of sodium and potassium is in accord with Mathews's theory, the stimulating power of the hydroxyl ion being great enough to overcome the depressant action of any cathion with which it may be associated, but the theory fails to account for the very opposite action of the ammonium hydrate.

d. The depressor action of the calcium and the magnesium salts on the heart muscle and the heart ganglion and the stimulating action of the sodium salts on the same tissues have already been described. We have also seen that the chloride of potassium de-

presses the heart muscle, while on the heart ganglion it has a primary stimulating action. These actions of the sodium, potassium calcium and magnesium salts conform to the demand of Mathews's theory, with the exception of the primary stimulating action of the potassium chloride on the heart ganglion. According to the theory, the potassium chloride is a stronger depressant than the chloride of calcium (Table XIV).

In addition to the above salts, the specific influence on the ganglion and on the muscle of the chlorides of rubidium, cæsium,

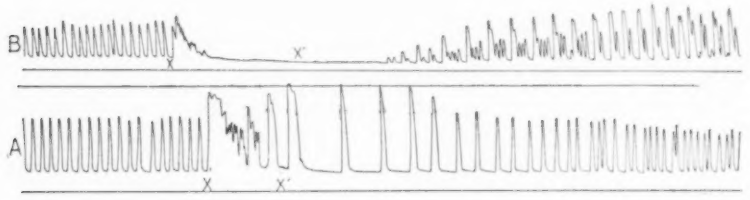

FIGURE 10. — About five-ninths the original size. Tracings from the anterior end of the Limulus heart. Ganglion isolated posteriorly. Anterior or recording end bathed in plasma. *A*, *x* = ganglion immersed in mixture of 10 parts isotonic NaCl to 1 part isotonic $BaCl_2$. *X'* = this solution replaced by plasma. *B*, *x* = ganglion immersed in mixture of 10 parts plasma to 1 part isotonic RbCl. *X'* = this solution replaced by plasma, showing primary stimulating action of these chlorides on ganglion.

barium, strontium, and the cyanides of potassium and sodium was also determined. The action of the cyanides on the heart tissues of Limulus is very peculiar. The results from the cyanides will be reported in detail later. The specific action of the other salts may be briefly summarized as follows:

The chloride of rubidium has a very intense stimulating action on the ganglion, while it depresses the heart muscle without primary stimulation. The stimulating action on the ganglion is evident in dilution of one part ($\frac{6}{10}$ *n*) of the chloride to forty parts of plasma. In solution of one part of the chloride to ten parts of plasma the ganglion is thrown into incomplete tetanus, and this is quickly followed by cessation of the rhythm (Fig. 10, *B*).

Strontium chloride depresses both the ganglion and the muscle without primary stimulation, but less strongly than does the calcium chloride.

Cæsium chloride is a weak depressant both on the ganglion and on the muscle (Fig. 2, *C*). It may be applied to the ganglion in isotonic

concentration without any definite injury. In no case did this salt produce a primary stimulation.

The chloride of barium is a very intense stimulant both for the ganglion and for the muscle. In isotonic concentrations this chloride sends the heart muscle into incomplete tetanus and tonus almost at once, while the ganglionic rhythm is stopped within a few seconds after a primary stimulation. The action of this chloride was mainly studied in dilutions with sodium chloride, because of the abundance of sulphates in the plasma and the sea water. Even at the dilution of one part ($\frac{6}{10}$ *n*) of the barium salt to ten parts of isotonic sodium chloride the ganglion is stimulated to a condition of incomplete tetanus, and quickly stopped unless the solution is immediately replaced by plasma or sea water (Fig. 10, *A*).

The chloride of lithium is a weak stimulant to the ganglion. Its action did not appear to differ materially from that of sodium chloride. The influence of the lithium chloride on the heart muscle was not determined.

When we compare these data with the demand of Mathews's theory of chemical stimulation and depression we meet the following discrepancies: We have, in the first place, the opposite action of the chloride of rubidium on the ganglion and on the muscle. The theory requires the cæsium chloride to be a stimulating salt and the chloride of lithium should be a depressant salt, while the very opposite is the fact for the Limulus heart tissues. According to the theory, the cathions Ba, Rb, and Na have almost the same depressing power, and in consequence their chlorides also practically the same stimulating power (Tables XIII, XIV). In my experiments, the chlorides of barium and rubidium are many times as powerful stimulants on the ganglion as the sodium chloride.

The difficulties in squaring the facts with the theory, in its present form, may be summarized as follows: 1. Some chemicals have an opposite action on the ganglion from that on the muscle (the acids, the chlorides of potassium, rubidium, ammonium, the hydrate of ammonia). 2. Some chemicals act as stimulants that, according to the theory, should depress (acids [ganglion], the chlorides of lithium and potassium [ganglion]). 3. Some chemicals act as depressors that ought to stimulate (chlorides of cæsium and rubidium [muscle], ammonium hydrate [muscle]). 4. The relative stimulating and depressing power of many of the chemicals is greatly at variance with the theoretical demands.

THE NUTRITIVE REQUIREMENTS OF THE BODY.

By FRANCIS GANO BENEDICT.

ALL wild animals, save those in captivity, select their own food, while the food of domestic and captive animals is provided for them. With the exception of the inmates of public and penal institutions, mankind in general chooses its own food. Since the selection of this food is determined by a number of factors, we find that people have formed certain so-called dietetic habits. Variations in geographical location, climate, and proximity to the sea result in wide variations in the nature of the available food materials. A seafaring race would naturally be expected to subsist in large measure upon fish, while those removed some distance from the coast would rely more upon agricultural products, and domestic and wild animals. Climate determines the length of the growing season, and in many instances the nature of the materials grown; consequently, inhabitants of tropical climates obtain fresh agricultural products for the whole year, while those of the temperate or more northern climates, affected by the short growing season, would rely more upon their previously stored food materials, or upon game that could be obtained during the more rigid climate of the winter. Facilities in storage and transportation, however, afford opportunity for the inhabitants of one region to obtain the food products of another region.

Aside from these grosser factors, affecting the dietetic habits, we have those depending upon the individual, of which age, sex, weight, muscular activity, and financial condition are the most important. The young, growing individual requires relatively more food than the adult, who no longer has to build, but only to repair, tissue. In general, women consume less food than men. Body weight is a not inconsiderable factor in determining the food consumption, but muscular activity modifies, probably more than any other one factor, the quantity of food utilized by man.

No one recognizes more than those who are experimenting with foods and dietetic conditions the great irregularity in the estimates of different individuals regarding the quantity of food they consume. Even those more than ordinarily skilled in a knowledge of the composition of food materials are found to be very unreliable in estimates of their own food consumption. People are designated as "light" or "hearty" eaters, but the conception is more likely to be based upon the bulk or volume of the food materials, and especially on the amount eaten at a particular meal, than upon the actual amount of protein, fats, and carbohydrates consumed per day.

It becomes necessary, therefore, to reject as distinctly unreliable mere personal impressions regarding food consumption, and consequently we must make an actual study of the food consumed before we can have any accurate knowledge on this point. Such a study may be made in a number of ways.

For example, it is possible to form an estimate regarding the food supply per capita from the budget of a country; but the various methods of preparing food for consumption, and too frequently its careless use at the table, involve such varying amounts of waste that it is a very difficult matter to deduce from statistics of food purchased the amounts of food actually consumed per capita. Hence we must consider studies based upon budgets as of only partial significance.

The most accurate method of determining the food consumption of groups or of individuals is to make a careful record not only of the food purchased, but also of the waste. If to these data we add the number of persons eating, and the number of meals taken, it is possible to compute, with considerable accuracy, the amount of food actually consumed per man per day. By use of compilations of analyses of food materials,[1] it is possible to compute with reasonable accuracy the amount of protein, fat, and carbohydrates as well as energy consumed by the individual. This constitutes what is known as a dietary study.

Dietary studies may be divided into two classes: First, those in which the selection of food is free, as is the case in general; and second, those in which the selection of food is restricted, as in public and penal institutions, in which the kind and quantity of food are pre-

[1] KOENIG: Chemie der menschlichen Nahrungs-und Genussmittel; ATWATER and BRYANT: U. S. Department of Agriculture, Office of Experiment Stations, Bulletin 28.

scribed; or those studies, more properly called feeding experiments, in which the food is determined upon previously.

A large number of studies of both of these classes have been made with very great care, not only in America, but in many places all over the world, especially in Germany, England, and France. As a result of these studies, much accurate information is now available regarding the amounts of protein, fat, carbohydrates, and energy ordinarily consumed by people of different classes, occupations, habits of life, muscular activity, etc. A by no means complete summary of such data is presented in Table I.

These data regarding food consumption by different classes of people in different parts of the world were taken practically at random from a large compilation of dietary studies.

From a consideration of the results of such studies two things at least are very evident: that, all over the world, people who can obtain such food as they desire use liberal rather than small quantities; and that, the more active or severe the work done, the larger the quantity of food eaten.

There is not, indeed, any exact uniformity in food consumption by people in any given conditions of living; but the variations are, in the main, not too wide to permit of deriving what might be termed the average food consumption for different classes. By taking into account the large amount of data available as a result of the studies referred to above, the following estimates have been obtained, as showing approximately the average food consumption of people under certain circumstances (See Table II.).

Attention is given here only to the protein and energy of the food, as these are the important factors of the diet. Sufficient experimental evidence has accumulated to show that the nutritive values of the fats and carbohydrates of food are almost proportional to the amount of energy that they can furnish, 1 gm. of fat being equivalent to about 2¼ gm. of carbohydrate. Consequently, it is only important that the quantity of either or both be such that their total energy, when added to that of the protein of the diet, shall supply the amount required by the body.

Distinction is made between the total and the digestible protein in the food consumed. The actual value to the body of any given diet as a source of any nutrient is measured not by the total quantity of such nutrient supplied, but by the quantity that is digested and absorbed.

TABLE I.

Subjects.	Protein.		Fuel value.
	Total.	Digestible.	
	grams.	grams.	calories.
Lumbermen, U. S.	185	170	6400
Chinese farm laborers, U. S.	144	132	3982
Weavers, England	151	139	3475
Mechanics, Sweden	189	174	4591
Italian brickmakers, Germany	150	138	4169
Lumbermen, Germany	130	120	6015
Farm laborers, Austria	159	146	5096
Athletes, U. S. (College)	175	161	4741
Professional bicyclists, U. S.	202	185	4960
Painter, U. S.	138	127	4486
Printer, U. S.	117	108	3554
Boxmaker, U. S.	131	121	3189
Carpenters, U. S.	119	109	3230
Masons, U. S.	112	103	3236
Chinese laundrymen, U. S.	135	124	3480
Laborers	130	119	3346
Farmers	100	92	3432
Machinists	102	94	3470
Factory operatives, Russia	119	109	3190
Mechanics, Germany	134	123	3061
Mechanics, Sweden	134	123	5331
Negro farmers, U. S. (Va.)	108	99	3657
Negro farmers, U. S. (Ala.)	58	54	2926
Business men, U. S.	98	90	2947
Professional men, U. S.	104	96	3143
Students, men, U. S.	110	101	3496
Students, women, U. S.	85	78	2696
Physician, Denmark	135	124	2786
Physicians, Germany	111	102	2511
Physician, France	92	85	1851
Professor, Japan	123	113	2343
Students, Japan	94	86	2712
Medical students, Sweden	127	117	2723
Poor families in New York, U. S.	75	69	2087
Patients in hospitals for the insane:			
Men, District of Columbia	93	86	2705
Men, New York	80	74	2480
Women, New York	64	59	2110

A certain portion of the ingested food passes through the alimentary tract unabsorbed and is excreted in the fæces. A large portion of

fæcal matter is not, however, undigested food. Recent investigations show that the material formerly considered undigested food really consists in large measure of residues from the digestive juices and epithelial débris from the intestinal wall, commonly designated "metabolic products." In spite of this fact, since we lack proper methods for distinguishing between undigested food and the metabolic products, experimenters quite generally consider the material of the fæces as pertaining directly or indirectly to the digestion of food, and determine the digestibility of a given ingredient from the amount consumed in the food and that excreted in the fæces.

TABLE II.

Subjects.	Protein.		Fuel value.
	Total.	Digestible.	
	grams.	grams.	calories.
People with active muscular work such as lumbermen, athletes, etc.	175	160	5500
People with ordinary muscular work, such as carpenters, farmers, laborers, etc. . .	115	105	3300
People with light muscular work, such as professional and business men, clerks, etc.	100	92	2700

Many experiments have been made to determine the digestibility of different food materials, and these show two things that are of much importance. The first is, that with vegetable foods as ordinarily prepared for the table, the protein and fats are somewhat less thoroughly digested than is the case with the protein and fats of animal foods; and the second that the body absorbs the nutrients from food in practically the same proportions without regard to the amount ingested and consequently, even if excessive amounts are ingested, they are in general well absorbed.

Regarding the first of these two points it may be said that about 97 per cent of the protein in animal foods, such as meats, eggs, and dairy products, is digested, whereas only about 84 per cent of the protein of vegetable foods as ordinarily served is digested. It has been found that with a diet containing both animal and vegetable foods, such as is commonly used in this country, about 92 per cent of the total protein is digested. This is the factor used in computing

the digestible protein in the food consumption by different classes as given in the preceding table. In the case of fats, at least 95 per cent of the animal fat is digested, whereas vegetable oils and fats appear to be somewhat less thoroughly absorbed; but since by far the larger part of the fat in our diet is obtained from animal food, the digestibility of the total fat in the diet is about 95 per cent. In the case of carbohydrates, such as sugar and starch, at least 98 per cent appears to be digested.

The maximum amount of energy that may be obtained by the body is not the total potential energy of the food consumed, since the potential energy of the corresponding fæces must be deducted. Furthermore, the body is not capable of completely oxidizing the absorbed protein, the incompletely oxidized products being excreted in the urine, and the energy eliminated in this way must also be deducted. The net or actual energy value to the body is the total potential energy of the food minus that of the corresponding fæces and urine. This is expressed in the tables as "fuel value."

Regarding the fact that food will be equally well digested and absorbed whether the amount eaten is large or small, it is essential to bear in mind that when food is absorbed in excess of the immediate needs of the body it will be stored, chiefly as body fat. The most familiar example of this is seen in the fattening of domestic animals. There is conclusive experimental evidence that what holds true in that case is likewise true for the human body. Since, then, by far the larger part of the ingested food is absorbed, and is either used immediately or stored in the body, the question arises, "What are the requirements of any given individual for food"? It remains, then, to consider to what extent the normal average food consumption of people of different classes corresponds to their actual physiological requirements.

Obviously food should be ingested in just the proper amount to repair the waste of the body; to furnish it with the energy it needs for work and warmth; to maintain it in vigor; and, in the case of immature animals, to provide the proper excess for normal growth, in order to be of the most advantage to the body. In just what proportions and amounts the various nutrients should be ingested, experimental physiology and studies of nutrition attempt to determine.

The food requirements may be said to consist of two probably distinct factors: first, the requirement for protein; and second, the requirements for carbohydrate and fat, or more specifically, the re-

quirement for energy. In the latter case, the energy value of the protein supplied must obviously be taken into consideration.

In this discussion normal nutrition and the physiological demands of the body only are considered.

Partaking of foods difficult of digestion, excessive amounts of sweetmeats and occasional excessive quantities of food commonly lead to disturbances in the alimentary tract. The organism tends to correct such excesses, however, by creating a disgust or distaste for similar foods upon subsequent days and there is, therefore, almost invariably a diminished intake.

The general question discussed here considers, however, not so much food ingested as food absorbed, and it is assumed that well prepared and digestible foods are consumed in quantities not too excessive so far as any particular meal is concerned, thus eliminating the question of periodic or occasional over-eating.

Protein Requirement.

Considering the adult individual and thus eliminating the question of growth, protein is absolutely required to repair the constantly wasting muscular and other protein-containing tissue. Recent attempts to measure the imperative demand for this specific purpose seem to indicate that the quantity of previously formed tissue protein broken down per day is relatively small. The researches of Folin[1], seem to point to the excretion of certain products other than urea, notably creatinin, as a measure of this tissue disintegration. Aside from the tissue protein, there is in the body a large supply of fluid protein not actually incorporated into the material of the living cell. To what extent this mass of fluid protein can be lessened is a very important point, but unfortunately experimental evidence is lacking. The matter is still further complicated in that thus far no perfectly clear method for determining this point has been devised.

In the case of the fasting individual the excretion of nitrogenous material is far in excess of the amount which, according to Folin, corresponds to disintegrated tissue protein. Indeed, the excretion of nitrogen during the first days of fasting is, in general, not appreciably less than when the subject is on his accustomed diet. As the fast progresses, the amount of nitrogenous material excreted slowly diminishes, but in experiments in which men have fasted thirty days, the

[1] Folin: This journal, 1905, xiii, p. 117.

amount thus excreted is always considerably more than the small amount probably representing normal tissue disintegration. It is of course possible that during fasting the organized tissue may be more rapidly drawn upon than during normal food ingestion, though the creatinin output is no larger. On the other hand, it seems highly probable that there is some demand for nitrogenous material in the organism other than that required to repair tissue waste. This assumption is far from precluding that there may be a minimum amount of nitrogenous material with which the needs of the body can be fully supplied.

It has already been pointed out that the average amount of protein consumed by men at sedentary occupation is not far from 100 gm. per day, and that this amount increases as the muscular work increases, being noticeably larger for hard work. It was formerly held by some physiologists that the energy for muscular work is derived wholly from the disintegration of nitrogenous material; but it is to-day commonly accepted that increased muscular work does not materially increase the disintegration of muscular tissue. It would therefore appear that the increase in the amounts of protein eaten by men at severe muscular work is not needed for the specific purpose of repairing excessive waste of protein tissue. Why, then, do we find this increased consumption of protein with increased muscular exercise, and why do people in general consume more protein than is probably required to repair waste tissue?

It is a very significant fact that these conditions are quite universal, as shown by statistics obtained in civilized countries in all parts of the world. Dietary studies made in England, France, Italy, and Russia, show that a moderately liberal quantity of protein is demanded by communities occupying leading positions in the world. This, contrary to popular impression, is true also of the Japanese, for the better class select diets with relatively liberal supplies of protein in proportion to the body weight. Dietary studies with Chinese on the Pacific coast show the same fact. It certainly seems more than a remarkable coincidence that peoples varying so widely as these in regard to nationality, climatic and geographical conditions and dietetic habits, should show such agreement in respect to consumption of protein and energy. Nevertheless, it has been frequently questioned whether the diet of mankind in general could not be very materially improved, with regard to the quantity of protein consumed.

Evidence on this most important point may be obtained in two

ways. First, the regular diet of animals, individuals, or communities may be experimentally so adjusted as to include a smaller amount of protein. The experiments may last from two days to several months, and are usually accompanied by observations as to the health, strength, and both mental and general conditions of the subjects. The second important source of evidence regarding the minimum protein content of the diet is that of dietary and sociological studies of persons, families, or communities who have lived for many years on a diet low in protein. This evidence is based on habits of life continued in many instances over years, if not, indeed, generations.

With regard to the first kind of evidence, it is of interest to consider first, experiments made on men, and later, those made on animals.

Experiments with men. — It has clearly been established by many observers, notably Hirschfeld,[1] Klemperer,[2] and Siven,[3] that nitrogenous equilibrium may be established with quantities of protein in the diet much less than those commonly consumed. In nearly all these cases the subjects have lived for varying periods of time, frequently weeks and months, as comfortably on the restricted diet as on the normal diet. Indeed, some have claimed very superior advantages in the way of improvements in health as a result of the low protein diet.

Of all the experiments heretofore made in which the low protein diet was used, none can compare with the exhaustive series of experiments recently completed by Professor Chittenden of New Haven.[4] In the Popular Science Monthly, of June, 1903, Chittenden tells us that his interest in the subject of minimum protein requirement was first aroused by the dietetic habits of Mr. Horace Fletcher. Several months before the investigation was undertaken, during a preliminary experiment in Chittenden's laboratory, Mr. Fletcher had subsisted on a diet which provided for an average daily protein metabolism amounting to but 41.25 gm., and his body weight, 165 pounds, remained practically constant.

A second experiment with Mr. Fletcher included a study not only of the intake and output of nitrogenous material, but likewise a com-

[1] HIRSCHFELD: Archiv für die gesammte Physiologie, xli, p. 533; also, Archiv für experimentelle Pathologie und Pharmacologie, cxiv, p. 311.

[2] KLEMPERER: Zeitschrift für klinische Medicin, xvi, p. 550.

[3] SIVEN: Skandinavisches Archiv für Physiologie, x, p. 91.

[4] CHITTENDEN: Physiological Economy in Nutrition, 1904.

parison of other food and excreta. Results similar to those of the first experiment were obtained. Mr. Fletcher furthermore maintained that he had lived upon a diet similar to this for the five years preceding. So far as the *protein metabolism* during the two experimental periods is concerned, the results obtained differ in no wise from those of other experimenters.

The elaborate series of experiments by Professor Chittenden previously referred to were made with three groups of individuals, namely, professional men, soldiers, and athletes.

Of all the studies made on professional men, that made by Chittenden on himself is by far the most remarkable. For over two years he has subsisted on a diet that is markedly lower in its protein content than was his former diet. He maintains that in respect to a rheumatic trouble and general physical and mental condition he is much better than on his former diet. Nowhere in the literature of nutrition do we find an experiment so painstaking and accurate, covering so long a period, and with a diet of so low a protein content following a normal diet. It has justly been cited as "a monument of fidelity." Of the other men, none reached so low an absolute nitrogen level as did Chittenden, but the weight of this subject is small, 57 kilos (with clothes), and consequently the absolute amount of nitrogen required by a person of his body weight would be less than that required by a larger subject; yet on the basis of per kilogram of body weight one of the men showed a slightly smaller metabolism of protein than did Professor Chittenden.

Professor Chittenden assures me that he still metabolizes no more protein than he did during the period of the experiment, and so far as the other men are concerned, I am further assured that while they all are excreting somewhat more nitrogen per day than during the experiment, their dietetic habits are still such as to include but a small amount of protein per day.

Although in certain respects these experiments are far superior to others, the results obtained are strikingly similar to those of previous observations. A number of other people have lived and maintained body weight and health on diets containing small quantities of protein. It has not yet been incontrovertibly proven, however, that such a diet could be most advantageously adopted for all time, nor that the apparent improvements were due entirely to the low protein. In Chittenden's experiment, for example, we must at least consider for a moment whether the marked improvement in general condition

may not have been due in part at least to mental suggestion; furthermore, it is a well known fact that a routine of life, however simple, if persistently continued, does result in a benefit to health. It is not at all difficult to conceive of the subject of an experiment living a more abstemious life as a result of a daily routine which includes the collection of urine and fæces, a record of body weight, and a constant supervision of the diet, with the occasional weighings and sampling of food. Such routine might account for much of the beneficial results of the experiment.

The studies with the thirteen soldiers from a hospital corps gave results very similar to those obtained with the professional men: the soldiers were able to live on a diet with a greatly reduced protein content, and showed marked improvement in general conditions. The question again arises, and in this case even more emphatically, to what extent the improvement in physical condition, strength, etc., is attributable to other causes than the diet. These men from the hospital corps had not been subjected to the rigid regulations of the trooper. When they arrived in New Haven, they could not be considered in first-class physical condition. They were there subjected to the most rigid routine regarding hours of rising, meals, exercise, etc., and specified quantities of food were furnished them. What group of men could be put for six months under the personal supervision of Dr. W. G. Anderson, the director of the Yale Gymnasium, and not be very materially improved in condition irrespective of high or low protein in the diet? This group of men were two hours each day in the gymnasium, and this exercise was followed by regulation drills. Indiscretions in diet so far as concerns periodic over-eating, irregularity and undue haste at meals, eating rich and indigestible foods, sweetmeats, etc., were absolutely impossible under the rigid routine. The collection of fæces and urine would naturally lead to a more regular attendance to these functions. When this regularity of life is taken into consideration, it is not surprising that the men all showed a marked body improvement comparable to the nearly uniform improvement resulting from a sanatorium regimen.

Some of the reported experimental data regarding the digestion of food by these subjects are worthy of more than passing notice. For one period of six, another of seven, and another of five days, the food of the soldiers was accurately analyzed, and the nitrogen balance determined. During these periods the kinds and amounts of food

eaten were practically identical for all the soldiers. Thus we may consider that a definite quantity of food was caused to pass through eleven different alimentary tracts. We should normally expect to find that, while slight differences in digestive power would be exhibited by the different alimentary tracts, in the case of eleven normal healthy men the quantities digested would be very much alike; or in other words, that the total quantity of nitrogen excreted through the fæces would be nearly the same in all cases.

In a large number of digestion experiments on normal healthy subjects, this has been found to be quite generally the case. Indeed, in the chemical laboratory of Wesleyan University, an extensive investigation into the nutritive value of cheese has shown repeatedly that with twenty-five or thirty men, the same food passed into the digestive tract is absorbed to about the same extent in all cases. As a result of forty-eight experiments in which the same food was administered to thirty different individuals, of the 40 gm. of nitrogen ingested during a three-day experiment, 4.7 gm. were recovered in the fæces. In a very large majority of the experiments the variation was but two or three decigrams either side of the normal.

If, on the contrary, we examine the data from Professor Chittenden's soldiers we find some striking abnormalities. During the six-day period from January 12 to 17, for example, when a total of 49.4 gm. of nitrogen were ingested by each subject, there were found in the fæces from the different subjects quantities of nitrogen varying from 4.45 to 12.10 gm., with wide variations from the average (8.46 gm.) in a majority of the cases.

Similarly during the period of seven days from February 29 to March 6 inclusive, when the total ingested nitrogen was 66.7 gm. per subject, the amount found in the fæces varied from 7.017 to 16.155 gm., and here again there were wide variations from the average (11.66 gm.) with a number of men. The third period, namely, the five days from March 28 to April 1, shows less irregularities, although with the diminished amount of ingested nitrogen, 43.1 gm. per subject, the nitrogen in the fæces still varied from 7.797 to 11.469, while the average was 9.75 gm.

One great difficulty in determining the outgo of nitrogen in the fæces is to obtain an accurate separation of the fæces to correspond with the food of a given period, but obviously, the longer the experimental period, the smaller the influence of the possible error in separation. These experiments, however, were from five to seven days'

duration, whereas the cheese experiments mentioned above were of but three days' duration, hence the error due to separation of faeces should not be any greater in the longer experiments. This factor must therefore be eliminated in a comparison of the results from these experiments.

It is clear, then, that with the subjects of the experiments reported by Professor Chittenden, the variations in digestibility of the same diet are very much more marked than is the case with the normal individual, and it is to be seriously considered whether the abnormally low protein has not resulted in some disturbance of the alimentary tract, which affects its power of absorbing either the protein of food or the nitrogenous materials from which the so-called metabolic products are derived, — a disturbance which is analogous to that which has been observed in experiments on animals, as is shown later. Unfortunately, the data with the professional men and with the athletes do not afford such comparison, since the different subjects did not partake of exactly the same quantities of foods.

The studies with eight athletes, representing different kinds of competitive sports, were in the main very much the same as those with the other two groups. A large number of dietary studies made with athletes have shown that this class consume rather larger amounts of protein than is found in the diet of men at ordinary muscular labor. The athletes studied by Professor Chittenden were able to reduce, by a very material proportion, the quantity of protein in their diet; at the same time they gained in strength (some of them enormously, as indicated by tests with the dynamometer) and won in intercollegiate games and other athletic contests. It is reported that all the men felt better and were convinced that the lower protein supply was beneficial. In considering the gain in strength of the athletes, we must wholly eliminate, in all probability, the influence of unaccustomed athletic training and routine which doubtless played so important a rôle in the case of the soldiers, and, in some measure, in the case of the professional men. These men were all reported to be in "training form" at the time they began using the changed diet.

From these experiments, then, much more than from either of the others, it would seem possible to infer that the improvement in physical condition was due to the change of diet. Yet even these do not indicate the result should the diet be continued indefinitely. The great interest taken in intercollegiate athletics, amounting as it frequently does to an almost passionate desire to win at any cost, would

lead us to suppose that dietetic alterations, producing such a marked increase in strength and ability as these men showed, would be hastily utilized in the efforts for general increase in athletic success. I am informed, however, that all of the men who underwent these tests have returned practically to their former diet, though it is asserted that in certain cases the experiments have slightly influenced the dietetic habits of the athletes. To be sure, marked changes in dietetic habits would not at first meet with the unanimous approval of all coaches, trainers, and players; yet, if they were convinced that decided improvement would follow from such changes, they would be unreasonable not to adopt them. The men in these experiments, on the other hand, were largely free from the constraint of coaches and trainers. If they were permitted during the height of the athletic season to follow the régime of the experiments, and if they derived so much benefit from the small diet, it is difficult to understand why these men should not have adhered more rigidly to this method of dieting when preparing for subsequent intercollegiate events, save that there was, in all probability, an irresistible craving for more protein food, — a craving that was unconsciously followed with the relaxation of the régime of the special diet training table. A more intimate knowledge of the supposed benefits arising from a low protein intake could not fail to influence the dietetic habits for some time, and it is more than probable that these men are at present consuming somewhat smaller quantities of protein than formerly.

The experiments with athletes are of unusual interest in connection with certain objections that have been raised to the presence of a liberal supply of protein in the diet. While the carbohydrates and fats are completely oxidized to carbon dioxide and water, and thus leave the body in these forms, the protein molecule is only partially oxidized, and as a result of its ingestion there is left distributed throughout all the tissues the products resulting from the partial oxidation, of which uric acid is a type. It is urged accordingly that some of the products have been shown to be poisonous, and others probably exercise a toxic influence. Again, the excretion of these bodies (chiefly urea) takes place in the kidneys, and it is asserted that when the quantities to be excreted are large, as is the case with a diet containing a large amount of protein, the kidney is strained to excrete the material.

As indicated by the quantities of uric acid excreted by the different subjects of these experiments before, and indeed after, the diminished

diet was taken, the athletes excreted by far the largest amounts per day. This observation is in accord with practically all observations on athletes, who, as a rule, are accustomed to eat large quantities of uric acid-forming foods, such as meats.

While it may be true that for people of middle age, with sedentary occupations and with the metabolic activities in a more passive state, the ingestion of excessive quantities of uric acid-providing food is unwise, it is difficult to believe that athletes are hampered in their life, activity, and grace of movement by gouty and rheumatic infirmities induced by the presence of the large amounts of uric acid in the system.

Knowledge regarding the origin of uric acid, and the causes for variation in its excretion in individuals subsisting on a diet free from uric acid-forming ingredients is as yet too incomplete to permit the formulation of conclusive theories regarding the possible injurious effects of uric acid on the normal adult with a metabolic activity which is ordinarily maintained by a diet containing meat.

Since the excretion of material through the kidneys is largest with athletes, we may here question as to whether the kidney is unduly strained in performing its normal functions of secreting urine. The lungs are capable normally of providing for the interchange of respiratory gases when the body is at rest or when it is at hard muscular labor for eight to ten or more hours per day. Why the kidneys should not normally provide for an increase in the elimination of urea or even uric acid without detriment is not, as yet at least, clearly shown.

It is certainly not the consensus of opinion of medical men that the excretion of urea does involve a strain upon the kidneys. Furthermore, it is highly probable that the total quantity of urine excreted when the urine is of normal specific gravity is without influence on the kidneys themselves. The recent experiments of Sollmann and Hofmann[1] in administering large amounts of water to typhoid patients show that the kidneys secrete large volumes of urine without in any way impairing their function.

It would be more reasonable to suppose that the excretion of a small volume of a more concentrated urine of high specific gravity would produce a greater strain on the kidneys; consequently the food and drink should be so adjusted in amount as to cause a urine of

[1] Sollmann and Hofmann: American journal of medical science, February, 1905.

normal concentration to be secreted rather than to diminish the volume and simultaneously increase the concentration.

A much more plausible contention for a reduced protein diet may ultimately be shown by careful studies of the action of the intestinal flora on different diets. However, until our knowledge of the effect on the body of the different kinds of protein is more complete, speculation regarding the optimum quantity in the diet must at best be somewhat hazardous.

Experiments in which men have been furnished diets low in protein, and especially Professor Chittenden's experiments, are of the greatest importance in studying the metabolism of protein in the body,— scientific research of the highest type, and for the most part defying criticism. The proposed immediate practical application of the results of such experiments to the diet of people in general, however, legitimately demands a more complete discussion of the subject and a consideration of evidence that has as yet been but insufficiently presented by the advocates of the dietetic reforms. In judging of the convincing value of the New Haven experiments, we must not overlook the point brought out by Dr. Levene[1] that "every one under observation was continuing the work which he had been in the habit of doing and which he was trained to do, and further it has to be noted that no individual worked to a condition of actual fatigue, that wear of tissues in fatigue is out of proportion to the amount of work performed."

Experiments with animals. — Numerous studies have been made on the effects of a diminished proportion of protein in the diet of animals, and the results of these are decidedly significant in a consideration of the problem of the nutrition of man.

For the present discussion, animals may be divided, according to the nature of their food, into three groups: carnivorous, or high protein consumers; omnivorous, or moderate protein consumers; and herbivorous, or low protein consumers. The digestive tracts of these various groups of animals are designed to render absorbable these various proportions of protein. In the carnivorous dog a large quantity of hydrochloric acid is found in the stomach to combine with the large amount of protein consumed, while with the herbivora, and especially ruminants, a long sojourn of the food in the alimentary tract gives opportunity for the more complete digestion of the somewhat slowly digestible materials of the food.

[1] LEVENE: Journal American Chemical Society, 1905, xxvii, p. 1455.

Three investigations, one by Munk,[1] one by Rosenheim,[2] and a third by Jägerroos,[3] have been made on the general question of the effect of reducing the protein in the diet of the dog. It is a very significant fact that in all three cases the dogs, in a relatively short time at the longest, reached such a state that they either died or were in such poor condition that they had to be fed a more liberal amount of protein to keep them alive. In the opinion of these investigators the low protein diet resulted in a loss of the power of absorption from the intestinal tract, caused apparently by a change in the condition of the epithelial cells, as well as by a diminished secretion of the digestive fluids.

Professor Chittenden has attempted to explain these disastrous results of low protein diets with dogs by maintaining that there were other causes for their disturbances, such as the monotony and nature of the experimental diet. In the absence of further evidence, however, it is not unreasonable to assume that the disastrous result following all experimental attempts to reduce the protein in the food of the dog is a logical argument against such reduction.

It has been stated that the digestive apparatus of swine is very closely comparable to that of man, the hog occupying a position between herbivorous and carnivorous animals. Several important investigations have been made in recent years regarding the effect of feeding swine on a low protein diet compared with a high protein diet.

The extensive investigations of Shutt[4] on "soft" pork show conclusively that when hogs are fed on a low protein diet, *i. e.*, Indian corn, the pork is of very inferior quality, while if the protein content of the diet is raised by the addition of skim milk, legumes, or other nitrogenous materials, the quality of the pork is very markedly improved. Furthermore, it is the consensus of opinion of Canadian pork packers that the intestines of a hog fed on a low protein ration tear more easily when the carcass is dressed than those of an animal fed on a high protein ration, that is, the intestines are not so firm. It is interesting to note that bad results, so far as the "softness" of the pork is concerned, also attended the ingestion of a diet excessively

[1] Munk: Archiv für experimentelle Pathologie und Pharmakologie, cxxxii, p. 91.

[2] Rosenheim: Archiv für die gesammte Physiologie, liv, p. 61.

[3] Jägerroos: Skandinavisches Archiv für Physiologie, xiii, p. 375.

[4] Shutt: Bulletin 38, Department of Agriculture, Ottawa, Canada.

high in protein. Evidently swine are unusually sensitive to marked alterations in the diet.

The observations of Shutt in Canada have been abundantly confirmed by those of Skinner[1] in Indiana who fed hogs on a low protein diet of corn meal. He states that this diet impaired the digestive capacity, which became more apparent the longer the diet continued. This diet resulted in "poor appetites, light bone, deficient development in valuable portions of the carcass and a general state of unthrift, as shown by the hair, skin, and hungry look of the animals." The addition of but one-sixth the amount of the ration in material rich in protein resulted in normal growth and a healthy condition.

It is natural to expect that a lowering of the protein in the diet fed herbivorous animals, *i.e.*, those accustomed to relatively low protein diet, would be accompanied by less marked disturbances, and the disturbances might not appear for a considerable length of time. A most remarkable series of experiments, which throw a most significant light on this important problem, has been in progress at the Experiment Station of the University of Minnesota, under the direction of Professor T. L. Haecker.[2] The expensive portion of animal feeding stuffs being the protein, Haecker's experiments were made to determine as accurately as possible to what extent this lowering of the quantity of protein may be carried out with safety and economy. I am indebted to Professor Haecker for the details of this investigation.

The experiments were made on a large scale. Two groups of cows with ten in each group were fed for a period of three years, one of them with a diet containing the normal proportion of protein, and the other with a much lower protein ration. At the end of the second year the ordinary observer could not detect any differences between the two groups of animals, so far as physical tone was concerned, though the cows on the low protein ration had lost somewhat in weight. During the first part of the third winter both groups did well, but by January 1 the cows receiving the low protein diet began to fail. By the 13th of March it was absolutely necessary to increase the proportion of protein because the cows were very thin in flesh and the coat was harsh in feeling, a very well-known indication of poor condition or under-nutrition.

[1] SKINNER: Indiana Agricultural Experiment Station, Bulletin 108.

[2] Unpublished results.

One of the most remarkable features of this investigation is the prolonged and persistent beneficial effects of the former feeding. This group of animals, accustomed to a relatively low protein diet, existed upon a diet in which the protein was markedly reduced for a period of two years without showing any deleterious effects; but during the third year an increase in the proportion of protein in the diet was imperative.

While unquestionably experimenters have in many cases greatly erred in drawing from experiments on animals deductions regarding man, especially in the fields of pharmacology and physiology, yet the evidence supplied by these experiments cannot be entirely disregarded in any discussion of the problem of the nutrition of man.

With the omnivorous animal, man, we would expect to find that any diminution in the proportion of the protein in the diet would not be accompanied by as sudden or marked disturbances as in the case of the dog, and it is indeed more than probable that men may subsist for six months or even longer on a low protein diet without serious disturbances of the alimentary tract or of nutrition in general. Yet the evidence secured on Professor Chittenden's soldier squad, consuming a uniform diet, points positively to an abnormality in the fluctuations in the amounts of fæcal nitrogen with the different individuals.

Evidence of dietary studies and sociological conditions. — The evidence of short experiments should be supplemented by studies of dietary conditions in which a low proportion of protein has been consumed for a number of years.

From the results of numerous dietary studies made with different classes of people living under different conditions, it has been observed that, whatever may be true of a few individuals, with communities a generally low condition of mental and physical efficiency, thrift, and commercial success is coincident with a low proportion of protein in the diet. The negro and poor white of the South, the Italian laborer of southern Italy, all partake of diets relatively low in protein. That their sociological condition and commercial enterprise are on a par with their diet, no one doubts. Nitti[1] expresses the conviction that the proportion of protein in a diet is one of the greatest determining factors in the productive capacity of a nation. Furthermore, it seems clearly established that when people accus-

[1] NITTI: The Economic Journal, 1896, vi., p. 21.

tomed to a low protein diet are fed on a higher protein plane, as is the case when the southern Italians come to America, their productive power increases markedly.

Aside from the dietary studies of communities, there is a considerable amount of evidence at hand on the power of the body to subsist on low protein diet in the form of digestion experiments and dietary studies with vegetarians, fruitarians, and nutarians. Those of Albu,[1] Caspari,[2] Voit,[3] and more recently those of Jaffa,[4] are of the most interest. In these experiments the observers found the subjects living on relatively low quantities of protein. It is more than a coincidence, and worthy of note, that in nearly every instance the subjects were men or women of small body weight, some of the women being unusually small.

Experimental evidence is rapidly accumulating to show that the quantity of food required has a very intimate relation to body weight. Furthermore, the actual amount of protoplasmic tissue as distinguished from fatty tissue in the body apparently exercises a marked influence on the food requirements. Certainly it is commonly conceded by physiologists that persons of small body weight consume much smaller amounts of food than are consumed by the average man.

This fact explains in part, at least, the statement frequently made that the Japanese, whose diet contains a large proportion of rice, subsist on a diet very deficient in protein. A most complete compilation of Japanese investigations upon the nutrition of man has very recently been published.[5]

An examination of this compilation shows that the better class of people in Japan do not subsist on an essentially low protein diet. When we take into consideration the small body weight of the Japanese, we find that their diet is fully as liberal in protein as is the ordinary diet of the American in moderate circumstances. This statement is likewise made regarding the jinrikisha men, who perform great feats of strength and on presumably a very low protein diet. In the

[1] ALBU: Berliner klinische Wochenschrift, 1901, pp. 647, 670; also Die vegetarishe Diät, Leipzig, 1902, p. 65.

[2] CASPARI: Ein, Biochemisches Centralblatt, 1903, ii, p. 144.

[3] VOIT: Zeitschrift für Biologie, xxv, p. 232.

[4] JAFFA: U. S. Department of Agriculture, Office of Experiment Stations, Bulletin 132.

[5] OSHIMA: U. S. Department of Agriculture, Office of Experiment Stations, Bulletin 159.

one complete study of a jinrikisha man found by Professor Oshima, the diet was characterized by having a large quantity (158 gm.) of protein. Two other studies are reported, in which, however, only the nitrogenous output in the urine of the men was determined. In the one case, the nitrogen excreted corresponded to a very large proportion of protein, and in the other case to a moderate amount, or specifically, in the one case there was an excretion of nitrogen in the urine amounting to 20.62 gm. per day, which corresponds to a metabolism of protein of about 130 gm., and in the other a quantity amounting to 11.17 gm. per day or 70 gm. of metabolized protein. The subject of the latter experiment weighed 54.4 kgms., whereas the one in the other experiment weighed 69.5 kgms. Furthermore, the amount of work done by the lighter man was markedly less than that done by the heavier subject.

Perhaps the most remarkable experiment on the influence of the amount of protein in the diet is that resulting from the extensive investigations of the Medical Bureau of the Japanese Navy in their effort to eliminate the disease beri-beri, which affected a large proportion of the men in the navy.

A battleship was detailed to make a long voyage, and a dietary study was made of the food consumed by the sailors, sub-officers and officers. On this voyage the number of cases of beri-beri was very noticeable among the marines, with but two cases among the better-fed officers. At the end of the voyage a commission was formed which investigated the problem very thoroughly, and which recommended that the food of the sailors be altered so as to include a much larger proportion of protein, and that improved sanitary conditions be insisted upon. This gave rise to the passage of the Naval Food Supply Act. Before this change, the diet of the Japanese sailor supplied 91 gm. of protein per day. As a result of this act, the present diet of the Japanese sailor supplies 155 gm., which, considering body weight, is certainly very much above the average protein in the food of the ordinary man.

Another battleship then made a voyage over exactly the same route as the first, and with the changes mentioned the proportion of cases of beri-beri was very low. Of the two factors which evidently influenced this decrease in the percentage of beri-beri cases, namely, the increase of the protein in the diet and the improved sanitary conditions, there was little doubt in the opinion of the commission that the former was of decided influence. It is worthy of note that

Osler[1] recommends that Americans visiting districts infested with beri-beri partake of a large proportion of protein food.

Contrary to current impression, the Japanese, at least in the navy, are not fed diets low in protein. As shown by the investigations summarized in Professor Oshima's report, the same might be said of the Japanese army.

From the foregoing considerations, it is clear that while men may for some months reduce the proportion of protein in their diet very markedly, and apparently suffer from no deleterious consequences, yet, nevertheless, a permanent reduction of the protein beyond that found to be the normal amount for man is not without possible danger. The fact that a subject can so adjust an artificial diet as to obtain nitrogenous equilibrium, with an excretion of nitrogen amounting to but 2 or 3 gms. per day, is no logical argument for the permanent reduction of the nitrogen in food for the period of a lifetime. Furthermore, it is a matter of common observation that during the period of growth a considerable proportion of nitrogen in the diet must be maintained, otherwise the growth is defective. After the body has attained its growth, there is a period of years during which a liberal supply of protein must in all probability be used. With declining years and less muscular activity, the amount of total food as well as of protein can probably be advantageously lowered.

We may note, then, that the following observations militate against the view that a material reduction of protein in the diet is desirable:

1. From the results of the digestion experiments with the reduced diet on soldiers, it is seen that abnormally low protein may affect the absorption of nitrogenous material from the alimentary tract.

2. Animals fed diets low in protein do not thrive so well as on liberal quantities.

3. Dietary studies all over the world show that in communities where productive power, enterprise, and civilization are at their highest, man has instinctively and independently selected liberal rather than small quantities of protein.

[1] OSLER: Practice of Medicine (New York), 6th ed., 1906, p. 252.

Energy Requirement.

While all evidence regarding the true protein requirement of the human body is based necessarily upon experiments of long duration, and while there is considerable speculation with many differences of opinion regarding the true amount of protein necessary to keep the body in the best condition, no such uncertainty need exist regarding the question of the energy requirement. Knowing that the law of the conservation of energy obtains in the human organism, it is only necessary to know the energy output per day to determine the energy requirement, for the energy output *is* practically the energy requirement. With the respiration calorimeter, a chamber in which a man may live from one day to two weeks, and by which the output of carbon dioxide, water, and energy, and also the intake of oxygen, can be accurately studied, it is possible to measure the total heat output of individuals of different size, age, sex, and degree of bodily activity. By means of a stationary bicycle with variable friction the amount of work performed can be accurately controlled.

As the result of a large number of experiments it has been found that when a person is asleep during the early morning hours, and especially if no food has been taken, both bodily activity and heat output are at a minimum. The maximum output thus far obtained was with a subject performing very severe work upon the stationary bicycle. Between these two extremes are varying amounts of heat output dependent almost directly upon the external muscular activity. From the results obtained it is possible to formulate with a considerable degree of accuracy the energy requirement of a man under ordinary conditions of occupation. The greatest difficulty in establishing any absolute standards of this nature would be in deciding upon the amount of muscular work actually involved in different occupations. There are, for example, various interpretations of the terms "light," "moderate," or "severe," when used to indicate the grade of muscular activity. Furthermore, different individuals would vary in the amount of other exercise, such as walking, outside of their regular occupations. Nevertheless, it is believed to be possible in many instances to formulate energy requirements for varying conditions with reasonable accuracy.

It has been found that with a man of average size, weighing 66 kgms., and at rest within the calorimeter, the energy requirement for twenty-four hours is not far from 2270 calories. The average indi-

vidual outside of the calorimeter, pursuing his ordinary course of life but not engaged in any occupation, would actually require more than this, because with a greater liberty to move about than was permitted the subjects of the experiments, the movements of the body would call for the utilization of more energy. The figures given above, then, may be considered as practically the lowest requirement under any ordinary circumstances.

Muscular activity will increase this amount very materially. A professional bicycle rider performed sufficient work upon a stationary bicycle to increase his heat output to 600 calories per hour during the time he was working. It would certainly be a very exceptional occupation that would require any such amount of energy during the whole of the working period; but it is not improbable that the requirement for some kinds of work should approximate this at intervals. Certain forms of athletic exercise, as, for example, rowing a race, call for large expenditures of energy; and professional bicycle riders in long races utilize enormous quantities of energy.

In connection with the establishment of a standard for protein much lower than that in common use, Professor Chittenden likewise proposes to lower very materially the standard for energy requirement. The first subject with whom Professor Chittenden experimented was Mr. Fletcher.[1] From his study of Mr. Fletcher's diet, Professor Chittenden came to the conclusion that Mr. Fletcher was able to hold his own with 1700 calories of energy. During the period of this experiment Mr. Fletcher was given the drastic exercises of the Yale University crew, and consequently was undergoing an unusually large amount of muscular exercise. Professor Chittenden bases his estimation of Mr. Fletcher's energy requirement upon the fact that during the period of six days in which a small quantity of food was taken into the body not only was nitrogen equilibrium maintained, but the body weight also remained practically unchanged.

It was the writer's privilege to have Mr. Fletcher for three days as a subject of an experiment with the respiration calorimeter. Mr. Fletcher is a man of athletic physique, and accustomed to considerable exercise; but during these three days he was under unusual conditions of muscular inactivity. No other subjects in the same kind of experiment in the calorimeter have been quite so inactive. He spent a large part of each day reclining very quietly upon a cot, or sitting reading, though he wrote a little on a small portable type-

[1] CHITTENDEN: Popular Science Monthly, June, 1903.

writer. On the first day, during which he was more quiet than on either of the others, the heat output was 1802 calories; and on the third day, when he moved about and wrote a little more than on either of the other days, the output was 2044 calories. The average output for the three days was 1896 calories, whereas the amount of energy actually derived from the food (the total potential energy of the food, less that of the fæces and the urine) averaged only 1357 calories. To make good this discrepancy, therefore, the subject would have to draw upon his body material for 536 calories, and the data of the experiments from which the gain or loss of body material is determined show that he actually lost body material sufficient to supply this amount of energy.

In view of these facts regarding the expenditure of energy by Mr. Fletcher when without exercise, and in the light of experience obtained with other subjects in the calorimeter regarding the increase in output of energy during muscular activity, it is certainly safe to assume that on the days in which Mr. Fletcher was taking the exercises of the Yale University crew, his energy output could hardly have been less than 3000 calories. Since the intake on these days was but 1700 calories, it is obvious that Mr. Fletcher did not hold his own, and body material must have been drawn upon to a considerable extent.

The 1300 calories representing the difference between the intake of energy as estimated in the food and the probable output during the excessive muscular exercise could be accounted for by the combustion of 150 gm., or about one-third of a pound, of body fat. In the course of a short experiment, *i. e.*, six or seven days, there may easily have been a loss of one or two pounds of body fat compensated by a gain of one or two pounds of water in the body, so that the body weight would remain unchanged. That a man in suitable physical condition would do this for a certain length of time is not to be doubted; that he could keep up such a draft upon body material indefinitely is not to be considered.

This affords an excellent illustration of one of the errors involved in the assumption that body weight is an index of a gain or loss of body tissue. Fat and water constantly replace each other in the tissues. It is invariably the case that when a subject changes from a normal diet to one in which carbohydrates are largely predominant there is a tendency of the body to retain water in the tissues. On the contrary when a change is made to a diet in which the fats largely predominate, the tendency of the body is to lose water.

The peculiar significance of these facts in the present case is that Mr. Fletcher's diet consisted in a large part of prepared cereal, maple sugar, and milk; so that, aside from the fat and protein in the milk, the diet was essentially a carbohydrate diet. Consequently there was a tendency for the body to retain water, and any loss in body fat might be more than counterbalanced by the gain in water to the tissues.

In discussing the results of his experiments, Professor Chittenden suggests that his own requirement for energy is about 2000 calories per day, which is somewhat higher than his estimate of the energy obtained from his diet. This amount might indeed be sufficient for a man of his body weight and muscular activity.

Professor Chittenden was undoubtedly influenced in basing his own energy requirement on a somewhat higher level than that computed from the food ingested, by the fact that it was very difficult to compute the energy values of a mixed diet containing such elaborate dishes, as, for example, lettuce-celery-apple salad and chocolate eclairs. While Professor Chittenden states that his estimations for the energy value were obtained from a bulletin published by the Department of Agriculture, he must have been seriously hampered in these estimations by the fact that the composition and energy value of *cooked* foods are but incidentally considered in this bulletin. Obviously, with mixed diets of this nature, the only accurate method of obtaining the true energy value is to burn samples of the food in a calorimetric bomb.

In the case of the other professional men studied the energy requirement would be, without any question, considerably more than it was estimated that they were getting from their food.

It would hardly be possible, for example, for a man of 70 kilos weight to live the active life of Professor Mendel, who was Professor Chittenden's associate in the experiments and one of the subjects, on less than 2800 or 3000 calories per day, while it is estimated by Professor Chittenden that Professor Mendel's energy requirement would be fully met with a diet furnishing 2400 calories.

With the soldiers the muscular activity would certainly call for a far greater amount of energy than the 2500–2600 calories suggested by Chittenden. Two hours' activity in a gymnasium in addition to the regular drills, to say nothing of the distance covered in walking during the day, would call for much greater amounts of energy than the 2800 calories computed as furnished in the food.

The muscular exercise taken by the group of athletes was in all probability considerably greater than that taken by the soldiers, consequently the energy requirement was likewise greater. That these men held their own for several months can only be explained on the assumption that on the days on which the diet was accurately analyzed and weighed the subjects partook of less food than usual. Indeed, Professor Chittenden points out that this certainly was the case with some of the subjects. Periodicity or rhythm in dietetic habits is an almost inevitable associate of experiments on the nutrition of man.

The statement that the energy ordinarily consumed by man in his food is considerably in excess of the actual requirement may reasonably be doubted. Indeed, such experimental evidence as has yet been accumulated tends to show that there is in general a very even balance between energy consumption and energy requirement.

It is maintained by some physiologists that even above the critical temperature there may be heat production independent of muscular activity. Experimental evidence is, however, lacking to demonstrate that this heat production is appreciable in proportion to the total energy output, even during fasting and muscular rest. It seems highly probable, therefore, that no appreciable amount of energy can be produced other than as a result of muscular activity.

Consequently, we may say that the energy requirement is that necessary for the voluntary and involuntary muscular activity. Ultimately the energy required for this muscular activity other than that converted into external muscular work leaves the body in the form of heat and a measurement of the heat output may correctly be taken as an indication of the energy requirement.

Of the internal or involuntary muscular work, that of maintaining respiration, circulation, muscular tonus, and digestive and peristaltic activity are the most important. Little evidence is at hand regarding the work of digestion, but with man, consuming as he does food which is for the most part readily absorbed, it is reasonable to believe that the work of digestion contributes but a small part of the total heat output.

Digestion experiments show that the percentage of nutrients absorbed does not vary materially even when large amounts of food are consumed. Approximately, nine-tenths of the food ingested is actually absorbed. If more food is eaten than is required, the material will be stored chiefly as body fat and the individual will increase in weight.

That the body weight is too large in many cases no one will doubt, but that too much food is eaten after the weight is once established is quite another matter. Since the food ingested is absorbed, and if not used, is stored, it follows that when the gain in weight ceases the food ingested is no longer excessive for the then prevailing muscular activity of the individual. In this discussion it is assumed that excessive amounts of food are eaten and the muscular activity has not been increased, for obviously with increased muscular activity the energy requirement becomes larger and the increased amount of food may be all used and hence can no longer be designated as excess. It is becoming more and more evident to experimenters that the amount of food *required* is not so much a function of the actual body weight, as of the mass of active protoplasmic tissue muscle; and hence fat may be considered as so much inert material, affording on the one hand a good resistant protection to cold and on the other proving a burdensome load to carry about.

It is probable that in the majority of cases where the body weight is excessive, the surplus tissue could be partially dispensed with to advantage by eating diminished quantities of food and drawing slightly but persistently on body fat. The delicate adjustment between food consumption and food requirement is not, however, to be trifled with. Marked changes in dietetic habits should only follow the prescription of a competent physician.

Since the law of the conservation of energy obtains in the human body, it follows that the energy requirement is the energy output and as was seen from the results of the experiment with the respiration calorimeter referred to, the deficiency in energy of the diet must be compensated for by a draft upon body material so long as the energy output exceeds the potential energy of the food. The energy of food can be lowered only when the energy output is lowered a corresponding amount. Since energy output is almost entirely determined by muscular activity, a lowered diet necessitates a lower plane of muscular activity to avoid a persistent draft upon body material.

In conclusion, while the experiments of Siven, Hirschfeld, Folin, Caspari, and more especially Chittenden, threw most important light upon the metabolism of nitrogenous material in the body, the evidence they present is far from sufficient to assert that the amounts of protein ordinarily consumed should be permanently and materially diminished. On the contrary, the evidence set forth here would favor the view that

permanent reductions are decidedly disadvantageous and indeed not without possible danger.

Furthermore, in the light of experimental evidence obtained with subjects in the respiration calorimeter and the fact that the law of the conservation of energy obtains in the human body, it follows that energy intake can be diminished only as energy output or muscular activity is diminished. These facts make any proposition to lower food consumption unaccompanied by decreased muscular activity impracticable.

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ON THE COMPARISON OF CONDUCTIVITY AND FREEZING POINTS OF SMALL QUANTITIES OF BODY FLUIDS IN HEALTH AND DISEASE.

By THOS. M. WILSON.

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I. Introduction.

THE objects sought in this investigation are twofold: The elaboration of an accurate method of finding the freezing point of small quantities of fluids from the human body and, secondly, a comparison between the conductivity and freezing point of such quantities in health and disease.

The freezing point of 10 to 15 c.c. of liquid can be readily ascertained by means of the ordinary Beckmann thermometer. But, it frequently happens in case of blood, animal secretions, contents of tumors and swollen joints, cerebro-spinal fluids, that less than 1 c.c. is available, so that the ordinary method of ascertaining the depression of the freezing point or $\triangle$ is not feasible. The importance of being able to determine the freezing point of small quantities led Dr. G. N. Stewart to make some preliminary experiments so as satisfy himself as to the feasibility of the problem.

He first tried to obtain the freezing point of blood by using a method similar to that which Hammerslag employed in obtaining its specific gravity. When a drop of blood suspended in an indifferent fluid freezes, the specific gravity of the drop decreases and consequently the drop will begin to rise. If the indifferent liquid were gradually cooled and its temperature taken at the point where the drop began to rise, this temperature would be the freezing point of the blood examined.

Three obstacles made this method unavailable:

1. The opacity of the side of the vessel used to cool the liquid rendered observations difficult. 2. The maintenance of a fixed temperature when freezing occurred. 3. The slow action of the drop after freezing.

Dr. Stewart tried mixing the fluid for examination with indifferent substances such as aluminium filings and blood, olive oil, and water. Table I gives results for two different combinations:

TABLE I.

18 c.c. benzol + 2 c.c. blood.	20 c.c. mercury + 0.4 c.c. blood.	
2.856	2.180	2.450
2.865	2.170	2.675
2.910	2.233	2.685
2.950	2.416	2.525

Small quantities of either mercury or benzol and salt solution, were next tried, — 1.6 c.c. mercury + 0.4 c.c. sodium chloride, and 1.6 c.c. benzol + 0.4 c.c. sodium chloride. The results obtained from these were not recorded but were of such a nature as to warrant a fair chance for the successful prosecution of the work. It was at this stage that the problem was given to me.

II. The Electrical Method of Freezing Small Quantities.

An electrical method was first tried. Differences in the temperature of a fine wire closely coiled and insulated was measured by means of a sensitive tangent mirror galvanometer. The aim sought was to have such an increased resistance in the coil so that when heated 0.001° C. it would cause a deflection of 1 mm. on the millimetre scale. The copper wire was coiled in the form of a bobbin on a capillary glass tube. It had one terminal of the wire penetrating the bore of the tube and coming out at the upper end, while the other terminal left the bobbin outside of the glass tubing. It was rendered impermeable to water by having been dipped into molten wax. The coil was then suspended alongside of a finely graduated thermometer and the two adjusted on an upright position in a large glass vessel containing about four quarts of water.

The coil was then connected with a Wheatstone bridge and its resistance measured at 18° C. by the aid of a telephone appliance. The value found was 0.69 ohm. This value was only approximately correct, since 0.01 ohm was the closest measurement which could be made with the Wheatstone employed. The fraction of an ohm beyond this was measured by the tangent mirror galvanometer. The method here was very similar to the ordinary method of measuring resistance with the Wheatstone bridge and telephone. In place of the induced current, as used in the former case, a constant current from a large

Daniell cell was employed while the telephone of the former case was replaced by the mirror galvanometer. The connections with the Wheatstone bridge were identical in each replacement.

The tangent galvanometer was arranged on a stand fastened directly to the stone wall and protected from all air currents. A telescope, with the millimetre scale attached, was placed about nine feet from the mirror. The results for small changes in temperature are given in Table II. The values in column 5 are calculated on the basis of a resistance of 15 ohms in the coil instead of 0.69 ohm of the bobbin used. The deflections for a large increment of temperature show that a deflection of 1 mm. corresponds to a change of 0.008 C. in the circuit.

In harmony with the above it was found that a deflection of 13 mm. on the scale was got by increasing the resistance in the circuit of the bobbin by 0.01 ohm. Now for an increase in 1° C. in copper wire the resistance increases 0.4 per cent, hence on a coil of 0.69 resistance an increase of 1° C. would be 0.00276 ohm (0.69×0.004), and on this basis 0.01 ohm would be the increase in resistance for 3.623° C. increase in the temperature of the coil. These corresponding values are given in Experiment 11, Table II, and are not far from the actual values obtained.

By using platinum whose specific resistance is 8.1 times that of copper, and a longer wire a coil, having a resistance of 15 ohms, can be wound into a cylinder 14 mm. long and 7 mm. in diameter. On the basis of this the calculated values are given in column 5.

Some theoretical considerations regarding the electrical method. — To fit such a cylindrical platinum coil, a fine glass tube could be selected having an inner diameter of 10 mm. Into this tube the coil could be suspended so as to almost touch its bottom and with 0.8 c.c. of fluid in the tube the coil would be completely immersed.

This reception tube containing the coil and fluid to be frozen could be suspended in a suitable freezing mixture. The stirring could be done by attaching the upper end of the suspended coil to the armature of a vibrating electro-magnet. The millimetre scale should next be standardized in hundredths of degrees with a total range of about 2° to 3° C. below zero. When about −0.4° C. was reached the investigator would inoculate the fluid by momentarily raising the bobbin from the liquid and adding a few flakes of snow to it. He would then quickly replace the bobbin and set it vibrating by means of the adjustment to the armature of the electro-magnet.

With the freezing apparatus arranged alongside of the telescope and with the fluid in the reception tube the observer would watch through the telescope the fall in the temperature of the liquid.

The observer would then notice the gradual deflection of light from the mirror on the scale as the liquid was showing cooling and then as freezing occurred the more rapid movement of the mirror in the

TABLE II.

No. of experiment.	Deflection in mm. as seen through telescope.	Changes in temperature of the water in the glass vessel in degrees centigrade.	Temperature corresponding to a deflection of 1 mm. when the total resistance was 0.69 ohms.	Calculation of temperature for 1 mm. deflection with total resistance of 15 ohms.
	mm.	c.	c.	c.
1	2.5	0.6°	0.24°	0.011°
2	2.5	0.9°	0.36°	0.016°
3	0.5	0.2°	0.4°	0.018°
4	1.5	0.6°	0.4°	0.018°
5	0.5	0.3°	0.6°	0.027°
6	1.9	0.95°	0.5°	0.022°
7	2.0	0.3°	0.15°	0.006°
8	3.0	1.5°	0.5°	0.022°
9	0.5	0.05°	0.1°	0.004°
10	17.8	3.35°	0.18°	0.008°
11	13.0	3.62°	0.277°	0.012°

opposite direction. When motion ceased the reading would be taken and its value gotten by referring to the standardized scale.

These considerations were not utilized since it was thought that a simpler method, better adapted for clinical purposes, could be found.

III. The Freezing of Small Quantities by the Beckmann Thermometer.

Throughout the rest of the experimental work on freezing small quantities the ordinary Beckmann was used. Gradually decreasing quantities of salt solution were frozen and the results compared with those from large quantities. Then, when a limit of accuracy was

reached, benzol or mercury was added. The benzol so readily formed an emulsion that it was soon discarded. Good results were, however, gotten with mercury.

Experiments were synchronously made regarding the best method of inoculation, the proper kind of stirrer, the most suitable freezing mixture and the number, thickness, and size of mantles, and the most suitable reception tube.

TABLE III.

Salt solution employed.	Mercury employed.	△ obtained.	Strength of salt solution.	Salt solution employed.	Mercury employed.	△ obtained.	Strength of salt solution.
c.c.	c.c.		gram.	c.c.	c.c.		gram.
5.0	....	0.451	0.128	0.7	....	0.454	"
1.0	....	0.453	"	0.6	....	0.450	"
1.0	....	0.452	"	0.6	....	0.457	"
0.9	....	0.450	"	0.6	....	0.463	"
0.9	....	0.449	"	0.6	....	0.455	"
0.9	....	0.458	"	0.3	1.2	0.465	"
0.8	....	0.455	"	0.3	1.2	0.453	"
0.8	....	0.459	"	0.3	1.2	0.462	"
0.8	....	0.463	"	0.3	1.2	0.459	"
0.8	....	0.450	"	0.3	1.2	0.459	"
0.7	....	0.451	"	0.3	1.0	0.464	"
0.7	....	0.457	"	0.3	1.0	0.453	"
0.7	....	0.453	"	..	....	....	....

Table III gives a few of the best successive results obtained. The freezing mixture was contained in a large vessel which telescoped loosely into a second vessel and between the two was placed sawdust. Finely divided ice and powdered potassium nitrate in excess was used as a cryohydrate, which gave a constant temperature of $-3°$ C. A freezing mixture of sodium chloride was found very unsuitable. The temperature was too low when the solution was saturated, and too variable when unsaturated, since the melting of the ice would decrease the degree of saturation and hence lower the temperature of the mixture.

Description of the apparatus employed. — Instead of a single mantle, as ordinarily employed in cryoscopy, two were used. The length of the reception tube was 15 cm., its outer diameter was 20 mm., its inner bore 14 mm., so that its thickness was comparatively great.

The stirring rod was of platinum 0.8 mm. in diameter, and its lower end snugly surrounded the Beckmann bulb, but was free to vibrate vertically 2.5 cm., with little friction between the bulb and reception tube.

The Beckmann was adjusted to a rubber cork so placed as to permit the lower end of the bulb to come within 2 mm. of the bottom of the reception tube. In the eccentric hole in the cover of the freezing mixture vessel was placed a fourth tube for the purpose of retaining the Beckmann during the process of inoculation. In case of spontaneous freezing of salt solution it was found that the process began from 1.7 to 3.8° C. below the freezing point of the fluid. This variability in the inauguration of the freezing process made a wide variation in results, where small quantities were examined. When a variable quantity of ice forms and the total quantity is small, then the fluid remaining would have a varying concentration, and hence its freezing point would also be variable. For this reason I employed an inoculation method which is described below.

A twisted strand of wires having a rosette at one end was used for inoculating purposes. The handle was so placed as to not allow the prongs of the rosette to touch the fluid in the reception tube. The few flakes of snow carried by its prongs could, however, easily fall from them into the fluid. The snow, got by shaving a small piece of ice, was contained in a porcelain vessel resting in a sodium chloride freezing mixture. Not more than half a grain of snow was required for each inoculation. At the beginning of each day's work the zero on the Beckmann scale was determined; the distilled water used was previously boiled and kept in a large, clean, tightly corked flask.

The technique of a freezing point determination. — The technique of taking the freezing point of a small quantity requires some description.

After preparation of the apparatus 1.2 c.c. of mercury was measured out by a clean graduated 1 c.c. pipette and transferred to the clean reception tube dried by compressed air. With another similar pipette 0.3 c.c. of salt solution was added to the mercury. The clean, dried Beckmann was then placed in the reception tube surrounded

with the two mantles. The cooling of the salt solution surrounded by the three tubes must always go on through at least two degrees, otherwise the results will probably be inaccurate. When a point was reached -0.4° C., the Beckmann was transferred to the fourth tube. This, being at a lower temperature, kept the mercury in the Beckmann from rising during the few seconds spent in inoculation. Upon replacing the thermometer it was almost invariably found that the mercury in the Beckmann scale had very closely regained the position it occupied before its removal from the fluid. Gentle stirring then followed at rate of three a second, and the scale closely watched with a jeweller's lens. The mercury kept sinking as in ordinary observations, only the under cooling was about 0.1° C. to 0.025° C.

Then a rise occurs which is maintained by gentle stirring for about thirty seconds, and after that with the same stirring the mercury on the scale will be lowered. In fact, one is surprised at how easily the readings are made. The time required for a determination after -0.4° C. is reached is about three minutes.

The rationale in obtaining the freezing point of 0.3 c.c. of salt solution. — The question arises as to the heat necessary to explain the phenomena observed in the freezing of 0.3 c.c. of salt solution. The bulb is 11.5 mm. in diameter and 24 mm. long. Assuming that it is all mercury its weight would be 33 gm. The specific heat of mercury is 0.0332, which gives a value of 1.09 calories to raise the bulb 1° C. If allowance is made for the glass and stirring rod, the results would not be materially affected (specific gravity of glass 3.3727; specific heat of glass, 0.198). In the complete freezing of 0.3 c.c. there is 24 calories of heat given off. If we assume that one half of this amount, 12 calories, goes to the salt solution, the stirring rod, the added mercury, and the reception tube, then the other 12 calories go to the bulb.

In stirring at the rate of three per second with a stroke of 2.5 cm., it was found that in the region of the zero the mercury sank at the rate of 0.1° C. per minute or 0.0016° C. per second (call this 0.002° C. per second). On this basis the bulb would be losing heat at the rate of 0.002 calorie per second, or, to stop this decline, 0.002 calorie per second must be supplied to the bulb. When the mercury in the bulb is under cooled 0.02° C. and rises to equal the freezing point of the liquid examined, and remains there for at least thirty seconds, the whole process, occupying say ninety seconds, would require

$90 \times 0.002 = 0.18$ calorie, besides less than 0.05 calorie for raising the mercury, the salt solution, etc., 0.025° C. These combined would yield a total of 0.23 calorie, or 2 per cent of the available heat.

When serum is used which contains about ten per cent of solids the heat available would be almost 10.8 calories, which would mean 2.5 per cent available heat required for a reading, hence the error would be noticeable.

The rejection of mercury in freezing blood and serum. — In regard to freezing blood and serum a difficulty arose which rendered the use of mercury a hindrance. An emulsion formed and very often no reading could be obtained. Table IV gives results when blood alone was used. It will be noted that the results are very accurate for 0.8 c.c., but for less than this the error is considerable. This was found to be due to the fact that a proportionately small part of the Beckmann bulb was covered with blood. The fact, too, that only the serum takes part in the freezing, or, more correctly, only the water of it, the 0.8 c.c. of blood, therefore, represents not more than 0.4 c.c. of fluid. When 0.6 c.c. of blood is used the results are very unreliable. From the table and from many results not here recorded the following rule has been deduced: Deduct 0.02° C. from the readings obtained for 0.7 c.c. of blood, 0.015° C. for 0.8 c.c., and 0.010° C. for 0.9 c.c., and for quantities slightly above these the readings will be very near the current results.

IV. The Improved Micagocrite and the Modification of its Use.

In a former paper[1] a small conductivity apparatus was described, which for brevity may be called a micagocrite. This instrument (since improved) was employed in all comparisons given in the tables which follow. It was discovered that the former micagocrite gave good results when used successively on one solution, but poor results when used successively on solutions of different strengths. The cause was in the presence of microscopic cracks, which had resulted in short circuits, and which had not been previously detected.

After several tedious trials in making new micagocrites, one was successfully blown which gave very accurate results. It was found that after measuring a 5 per cent potassium chloride solution which gave a resistance of 596 ohms, then washing off with distilled water, the resistance ran up beyond the limits of measurement by the

[1] Wilson: This journal, xiii, p. 139.

Wheatstone bridge used. On drying, by means of a fine drawn out pipette and compressed air, and refilling the tube, the resistance of 5 per cent potassium chloride was again 596 ohms. This simple but

TABLE IV.

Blood examined.	Quantity.	△	Blood examined.	Quantity.	△
	c.c.			c.c.	
Dog 1	6.0	0.592	Dog 3	0.7	0.566
"	0.7	0.609	"	0.6	0.585
"	0.7	0.599	"	0.6	0.577
"	0.7	0.605	"	0.6	0.573
"	0.7	0.603	"	0.6	0.577
"	0.7	0.609	"	0.5	0.627
Dog 2	12.0	0.632	Dog 4	10.0	0.610
"	15.0	0.664	"	10.0	0.612
"	0.5	0.644	"	2.0	0.616
"	0.5	0.668	"	1.0	0.613
"	0.5	0.660	"	0.9	0.620
"	0.5	0.648	"	0.9	0.623
"	0.5	0.658	"	0.9	0.619
Dog 3	6.0	0.558	"	0.8	0.624
"	6.0	0.556	"	0.8	0.615
"	1.0	0.560	"	0.8	0.618
"	1.0	0.562	"	0.7	0.624
"	1.0	0.558	"	0.7	0.627
"	0.9	0.556	"	0.7	0.630
"	0.9	0.554	"	0.7	0.636
"	0.8	0.577	"	0.7	0.615
"	0.8	0.559	Dog 4 (serum)	0.7	0.620
"	0.8	0.560	"	0.7	0.623
"	0.8	0.562	"	0.7	0.618
"	0.7	0.567	"	0.7	0.614
"	0.7	0.572	"	0.7	0.624

effective test was used about once a week and has shown no variation since it was made about four months ago.

Another change in technique was adopted. When the Beckmann thermometer was placed in the aluminium tube,[1] and the latter surrounded by pounded ice, it was found that twenty-five minutes was required to get a minimum temperature. When, however, the readings were taken at 18° C. in a copper tube, the minimum temperature was reached in eight minutes. For this obvious reason the conductivity readings were not taken at 0° C. as formerly, but at 18° C.; the results, however, are expressed at 5° C., as in my previous paper. The constant for 5° C. is got from the constant at 18° C., by reducing the latter 26 per cent; that is, for each degree that 5° C. is below 18° C. the conductivity will be reduced 2 per cent.

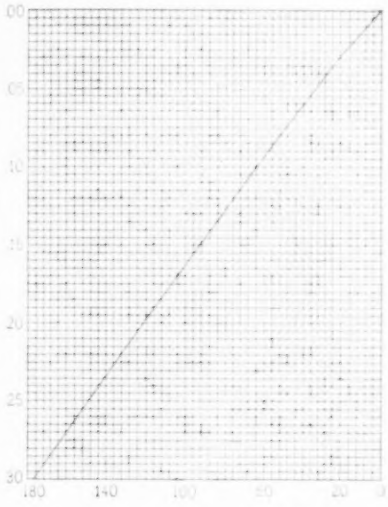

FIGURE 1.—Curve one for λ_5NaCl.

A tube of the same length as the hæmatocrite sealed at one end, and containing 0.43 c.c., was employed, instead of the one used in the previous work on conductivity. This change was due to the larger quantities required in freezing point determinations.

V. MEANS TAKEN TO VERIFY CONDUCTIVITY RESULTS.

In order to have a means of verifying results in conductivity, and of getting a relation between percentages of salt solution and conductivity, different strengths of salt solution[2] were prepared and the conductivities obtained by the micagocrite, compared with those calculated from Kohlrausch's tables. Table V gives these calculations and experimental results. From columns 1 and 3 a curve is plotted (Curve 1), the abscissa representing conductivities expressed

[1] WILSON: *Ibid.*, p. 145.

[2] KOHLRAUSCH and HOLBORN: Leitvermögen der Electrolyte, p. 158, 1898.

in reciprocal ohms at 5° C., while the ordinates represent the gram-equivalent of the sodium chloride.

TABLE V.

1	2	3	4	5	6	7	8
Equivalent of sodium chloride.	$\kappa_{18}10^4$= specific conductivity at 18°C.	$\lambda_5 10^5$ conductivity in reciprocal ohms at 5° C. × 10^8.	Sodium chloride in 100c.c. of solution.	$1000\,\eta$ = gram-equivalents of sodium chloride in column 4.	$\lambda_5 10^5$ calculated from curve 1.	Values obtained for sodium chloride in column 4 by micagocrite.	Per cent of error from columns 6 and 7.
grams			grams				
1.0	744.0	510.0	5.1714	0.854	465	458	−1.5
0.5	404.0	281.0	2.0	0.361	200	203	+1.5
0.3	255.0	177.0	1.25	0.213	134	136	1.0
0.2	176.0	122.0	1.0	0.170	101	107	6.0
0.1	92.5	64.0	0.91	0.155	93	98	5.0
0.05	47.9	33.4	0.75	0.128	76	78	2.0
0.03	29.7	20.7	0.50	0.085	53	57	2.0
0.01	20.0	13.9	0.25	0.062	28	29	3.0
0.01	10.2	7.1	0.083	0.014	10	10	0.0
0.0001	1.09	0.7	....	....	....	....	..

VI. The Means taken to Verify Freezing Point Determination and the Plotting of Certain Curves.

It was also thought advantageous to have the values of freezing point for the same solutions as are given in Table V. This relation of 1000 η to freezing point is based on knowing the gram-equivalent of the solution and the percentage of ionization.

Table VI gives the estimated experimental values in relation to the freezing point for the same solutions as in Table V.

From columns 1 and 3 is plotted Curve 2. The abscissas of this curve give the per cent of ionization, the ordinates the gram equivalent.

Curve 3 is plotted from columns 1 and 4, the abscissas of which gives the freezing point, and the ordinates the gram-equivalent. The differences in column 12 are no more than could be expected,

since the freezing points are based on ionization at 18° C., and the readings from the freezing point are taken below zero.

FIGURE 2. — Curve two for per cent of ionization.

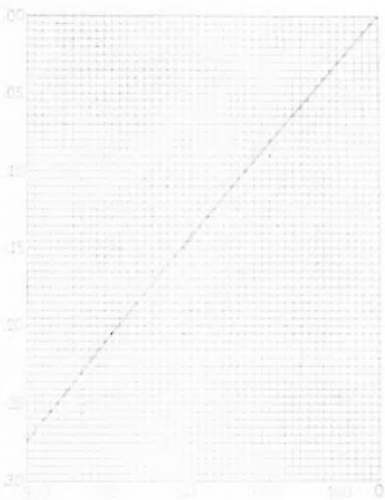

FIGURE 3. — Curve three.

VII. The Effect of Non-electrolytes on Conductivity.

In taking the conductivity of fluids where non-electrolytes are present in abundance, the question arose as to the depression which these had upon conductivity. Solutions of egg albumin, cane-sugar, and urea, each 2 per cent in strength, were prepared. Then likewise a second series, but in addition to the 2 per cent of the non-electrolytes sodium chloride was added so as to form 0.5 per cent of the mixture. Their conductivities at 5° C. and their freezing points were then determined, and the results tabulated as shown in Table VII.

The depression in conductivity at 5° C. is a little over 2 per cent in case of egg albumin, 1 per cent in the case of cane sugar, and 0.23 per cent in case of urea.

VIII. Some Results on Blood from Healthy Individuals.

The next table gives results of work done on human blood. It was shed from the vena mediana cubiti under asceptic precautions by

TABLE VI.

1	2	3	4	5	6
$1000\,\eta =$ gram-equivalent of sodium chloride.	$\frac{\kappa}{\eta}$ = equivalent of conductivity at 18° C. of solutions in column 1.	Ionization found by multiplying the values in column 2 by 100 and dividing by 110.3.	$\triangle$ values of same solutions calculated from values in columns 1 and 3.	Sodium chloride in 100 c.c. of solution.	$1000\,\eta =$ gram-equivalents of sodium chloride in column 5.
		per cent		grams	
1.0	74.4	67.4	3.097	5.0	0.854
0.5	80.9	73.3	1.603	2.0	0.341
0.3	85.2	77.2	0.938	1.25	0.213
0.2	88.2	79.8	0.665	1.0	0.170
0.1	92.5	83.8	0.360	0.91	0.155
0.05	95.9	86.9	0.172	0.75	0.128
0.03	98.3	89.1	0.104	0.50	0.085
0.02	100.2	90.8	0.070	0.25	0.042
0.01	109.7	93.2	0.035	0.083	0.014
At infinite dilution	110.3	100.0	....	....	....

[1] HAMBURGER: Osmotischer

means of a hypodermic syringe of about 1 c.c. capacity. The blood was transferred to a crucible and defibrinated by stirring with a small glass rod. Both the fibrin, which helps to increase the volume, so important in freezing small quantities, and the defibrinated blood were placed in a small glass tube and hermetically sealed. In this way it was conveyed to the laboratory and kept in ice until examined. The determination was usually made within forty-eight hours after shedding. The results for blood from healthy individuals are given in Table VIII.

The micagocrite tube employed for obtaining the hæmatocrite readings was inserted in the hæmatocrite frame and attached to a centrifuge which was propelled by an electro-motor at a speed of 75 revolutions per second for a period of eight minutes.

The reason for column 8 of this table will be made clear later on.

The reasons for giving columns 8, 9, and 11 will next be discussed.

TABLE VI.

7	8	9	10	11	12
Ionization obtained from curve 2.	△ values of solutions in column 5 got from curve 3.	△ from curve 4. The values are from Hamburger.[1]	△ from columns 6 and 7.	Cryoscopic values by Beckmann thermometer.	Error between columns 11 and 8.
per cent					per cent
69.2	....	....	2.673	2.557	....
76.2	1.114	....	1.113	1.184	7
79.2	0.684	....	0.706	0.717	4
80.8	0.573	0.588	0.571	0.603	5
81.3	0.523	0.537	0.520	0.541	2
82.2	0.440	0.445	0.463	0.453	2
86.6	0.293	0.300	0.291	0.303	2
87.7	0.149	0.145	0.148	0.152	2
93.2	0.050	0.050	0.051	0.52	4
....	....	....	....	....	....

Druck, 1902, i, p. 83.

IX. The Theoretical Consideration of the Composition of Human Serum.

For the purpose of this research it is necessary to examine the constituents of the serum, which make up the normal conductivity. Keeping in mind the facts known about the substances which enter into the conductivity of serum, Table X has been prepared.

Hamburger[1] gives in his table the oxides in 100 c.c. of serum as 0.95 per cent. Hammarsten[2] states that 60 to 70 per cent of the inorganic salts in serum is sodium chloride, and that about 1 per cent of the serum is inorganic salts. Of the electrolytes[3] in serum sodium chloride represents 63.4 per cent. The sodium chloride content in

[1] Hamburger: Osmotischer Druck, 1902, i, p. 437.

[2] Hammarsten: Physiologische Chemie, 1904, pp. 156–160.

[3] Bugarsky und Tangl: Pflugers Archiv, lxxii, p. 531.

Table X is placed at 0.585 per cent, whose conductvity forms 64.9 per cent of the total.

Jaksch [1] gives the alkalinity of the blood as 0.30 to 0.33 per cent. Straus [2] gives it as 0.43 per cent in terms of sodium carbonate. The

TABLE VII.

1	2	3	4	5	6	7
Symbols for solutions.	Grams in 100 c.c. of solutions of substances used.	Conductivity at 5° C. multiplied by $10^5 = \lambda_5 \times 10^5$	$\lambda_5 \times 10^5$ of A + B = conductivity of the non-electrolyte and 0.5 per cent sodium chloride.	$\lambda_5 \times 10^5$ of A + B − C = depression in conductivity due to the presence of the non-electrolyte.	$\frac{100\,A + B - C}{2\quad A + B}$ This formula gives the percentage depression in conductivity at 5° C. due to per cent of B.	△ values. The sum of the △ values of A + B equals that for C.
A	0.5 % NaCl	58.0	....	....	....	0.308
B	2 % albumin	9.8	67.8	3.0	2.2	0.058
C	2 % albumin + 0.5 % NaCl	64.8	....	....	....	0.364
A	0.5 % NaCl	58.0	....	....	....	0.308
B	2 % cane sugar	1.04	59.0	1.2	1.0	0.118
C	2 % cane sugar + 0.5 % NaCl	57.8	....	....	....	0.423
A	0.5 % NaCl	58.0	....	....	....	0.308
B	2 % urea	0.69	58.69	0.29	0.23	0.631
C	2 % urea and 0.5 % NaCl	58.4	....	....	....	0.927

larger part (63 per cent) of the alkalinity is due to non-diffusible bodies, which unite with the proteids and do not ionize. The rest of the alkalinity is diffusible, and is represented chiefly by sodium carbonate, sodium bicarbonate, and disodium hydrophosphate.

There is about 0.5 to 0.9 per cent of ammonia present in blood, but this is almost wholly united to the proteids, for if it were in the ammonia form, it would unite with water, and ionize, forming negative

[1] JAKSCH: Pflugers Archiv, 1876, xiii.

[2] HAMMARSTEN: Physiologische Chemie, 1904, pp. 156–160.

hydroxyl ions. But it has been shown that serum contains only a few more hydroxyl ions than is present in the purest water, so that we may neglect the ammonia of the serum.

Hammarsten [1] says regarding the actual percentage of alkali present in serum, that little is positively known.

In the table the bodies which can cause alkalinity by titration methods are given as 0.175 per cent sodium carbonate, 0.025 per cent sodium bicarbonate and 0.05 per cent disodium hydrophosphate. Sodium carbonate is given in largest amounts because in the lymph it is found to be 37 per cent of the salts present, these three alkalies represent a total of 0.222 per cent in terms of sodium carbonate. The total amount of phosphorus present in the salts of serum is represented in 0.05 per cent disodium phosphate.

There is only one other important element to be considered, namely, potassium. The error will be very slight if we assume that it is all present as the chloride. An estimation of the amount of potassium chloride which can be present shows that it cannot be more than 0.03 per cent.

Below is given an analysis of the five chief elements found in the salts of serum.

An approximate analysis of the conductivity of human serum. — In column 1, Table X the symbols A, B, C, D, and E represent the salts of sodium and potassium. The remaining salts of serum are represented by symbol F. Symbol G gives the total percentage weight of the salts in the table in terms of sodium chloride.

In column 3 are given the estimated percentages of the salts of serum. The value F in terms of sodium chloride represents the salts of calcium, magnesium, iron, manganese, copper, etc.; all these amount to a very small part of the whole and the error will be very slight by representing them as equivalent to 0.015 per cent of sodium chloride.

Before commenting on this table it is well to consider the freezing point of serum. It should, however, be noted that the total weight of the salts is 0.880 per cent, and this in terms of sodium chloride gives a conductivity of 90 while all the salts in Table X give 98.5.

An approximate analysis of the freezing point of serum due to its organic constituents. — It is hopeless to make an exact analysis of freezing point for normal serum but an approximate estimation can be made. The values of the percentage composition of serum given

[1] HAMMARSTEN: *Loc. cit.*

TABLE VIII.

1	2	3	4	5	6
Number.	Source of blood.	$\lambda_b \times 10^5$ = conductivity of blood at 5° C. got by micagocrite.	$\lambda_s \times 10^5$ = conductivity of serum at 5° C.	p = percentage of serum by formula $p = \frac{\lambda_b}{\lambda_s}(174.5 - \lambda_b)$[1]	h = per cent of serum got by using micagocrite tube in place of the ordinary hæmatocrite tube.
1	Healthy student	38.6	84.4	61.9	54.7
2	"	56.1	86.7	61.7	52.9
3	"	32.7	83.3	55.5	48.9
4	"	35.2	76.9	62.6	56.0
5	"	32.1	81.7	55.9	50.7
6	"	35.4	80.2	61.3	85.5
7	"	38.0	84.4	61.6	55.5
8	"	33.6	83.5	56.6	53.0

[1] STEWART: Journal of phy-

below are based on facts obtained from Stewart, Hammarsten, and others. A knowledge of the grams present per litre, the molecular weight of the organic body, and the fact that one gram-molecule of a non-electrolyte will depress the zero 1.85° C. enables one to estimate the freezing point for such organic body.

TABLE IX.

Elements.	Calculated from a chemical analysis of serum by Hamburger.[1]	Calculated from values given in Table X.
Chlorine	0.375	0.369
Sodium	0.326	0.326
Carbon	0.026	0.023
Potassium	0.018	0.016
Phosphorus	0.014	0.011
	0.759	0.745

[1] HAMBURGER: *Loc. cit.*, p. 487.

TABLE VIII.

7	8	9	10	11
$f - h$ Difference between columns 5 and 6.	$1000\,\eta$ = gram-equivalent of sodium chloride corresponding in conductivity to the serum values in column 4 when the latter is increased 7 per cent. The values are got from curve 1.	$\triangle$ for the values in column 8 got from curve 4.	Cryoscopic values by Beckmann thermometer according to method already described. The corrections for quantity given on sheet 3 are included in the figures here given.	$\triangle$ due to organic bodies = column 10 − column 9.
7.2	0.143	0.499	0.567	0.068
8.8	0.167	0.505	0.572	0.067
6.6	0.141	0.482	0.560	0.078
6.6	0.135	0.438	0.543	0.105
5.2	0.140	0.486	0.559	0.063
5.8	0.136	0.473	0.561	0.088
6.1	....	....	...	....
3.6	0.141	0.492	0.567	0.075

siology, 1899, xxiv, p. 356.

This Table XI shows a total freezing point of 0.076 for the organic constituents. The amount varies slightly, but probably in health does not go beyond this figure by more than 0.01° C. to 0.015° C.

An approximate analysis of the inorganic freezing point of serum. — Gases in 1000 c.c. of serum and their $\triangle$ values are as follows:

$O_2 = 0.26$ per cent = 2.6 c.c. per litre = 0.0002° C.
$N_2 = 1.8$ per cent = 1.8 c.c. per litre = 0.0015° C.
$CO_2 = 3.5$ per cent = 3.5 c.c. per litre = 0.0028° C.
0.0045° C.

The $\triangle$ for gases in solution is got from expression

$$\triangle = 8 \times 10^{-5}$$

where X = c.c. of gas present per litre and $\triangle$ is expressed in thousandths of a degree centigrade.

Table XII gives an analysis of the same salts as in Table X with respect to their relation to $\triangle$.

Column 7 gives a summary of the values in two separate figures. Here we notice that the total sum is 0.503. In this sum of 0.503 are included the gases in solution. The value for the freezing point for gases in solution can be gotten from the expression $\triangle = 8 \times 10^{-5}$

TABLE X.

1	2	3	4	5	6	7	8
Symbol.	Substance.	Grams per 100 c.c.	Gram-equivalent = 1000 η. The equivalent concentration η = gram-equivalent divided by 1000.	Equivalent conductivity at 18° C. = Λ.	Specific conductivity $\times 10^6 = \eta \times \Lambda \times 10^6$.	A contrasted with B + + F.	$\Lambda_5 \times 10^5$ value of B + C + D + E + F when A = 64.
A	NaCl	0.585	0.100	92.5	92.5	92.5	64.0
B	Na_2CO_3	0.175	0.032	86.0	27.5	....	....
C	$NaHPO_4$	0.050	0.007	96.0	6.7	....	....
D	$NaHCO_3$	0.025	0.003	100.0	3.0	....	....
E	KCl	0.030	0.004	126.0	5.0	44.2	30.5
F	NaCl for salts of Ca, Cu, Mn, etc.	0.015	0.002	82.5	2.0	....	....
				For gases			4.0
						Total	98.5
G	NaCl	0.880	0.150	....	....	....	90.0

where x is the number of c.c. of gas dissolved in one litre of solution at 0.760 mm. and 0° C. The mathematical basis for this expression need not be considered here.

If the value of 0.556 is taken for normal freezing point, and if 0.076 is the freezing point for the organic part of the serum, then 0.490 will be the freezing point for the inorganic, hence our value of 0.503 is too great by a little over 2 per cent (0.503 + 0.076 = 0.579).

The practical application of the above analysis of serum. — The average value for the conductivity of serum in Table VIII is almost 82. It must, however, not be forgotten that the conductivity of serum is less than it would be were no proteids present in it. It was found from Table VII that egg albumin depresses the conductivity of serum

TABLE XI.

1 Organic body.	2 Grams in 1000 c.c. of serum.	3 Molecular weight.	4 △
Proteids	7.5	10000 +	0.006
Glucose	1.5	180	0.015
Cholesterin	0.2	372	0.001
Lecithin	1.7	807	0.004
Fatty acid	0.6	256	0.004
Uric acid	0.1	156	0.001
Fat, neutral	6.5	755	0.016
Creatin	0.3	113	0.005
Urea	0.5	60	0.015
Lactic acid	0.4	70	0.009
Total			0.076

TABLE XII.

1 Sign.	2 Substance.	3 Grams per 100 c.c.	4 1000 η = grams equivalent.	5 Per cent of ionization.	6 △	7 Sum of B + C + D + E + F compared to A
A	NaCl	0.585	0.1	86.2	0.346	0.346
B	Na_2CO_3	0.175	0.032	70.0	0.100	....
C	$NaHCO_3$	0.025	0.003	90.0	0.010	....
D	$NaHPO_4$	0.050	0.007	95.0	0.024	....
E	KCl	0.030	0.004	94.0	0.013	....
F	NaCl for the salts of Ca, Mg, Fe, etc.	0.015	0.002	90.0	0.006	0.157
				Gases . .	0.004	....
				Total		= 0.503
G	NaCl	0.880	0.150	....	....	0.519

by 2.2 per cent for each gram in 100 c.c. of solution, so that on this basis the 82 would become 96 if there were not 7 per cent of proteid present.

The value of Table X is 98.5 which, like the value we got for inorganic freezing point, is a little over 2 per cent of the real value.

In other words, if the total quantity of salt were reduced 2 per cent and the reduction were made proportionately for all the salts, then the altered values would represent very closely the composition of the inorganic constituents of serum taken from healthy individuals. The freezing point would be 0.492, its conductivity 88.6. The total quantity of salts would be 0.880 per cent. If the values in Table XI were added the mixture would have a freezing point of 0.566 and a conductivity of serum of 82.

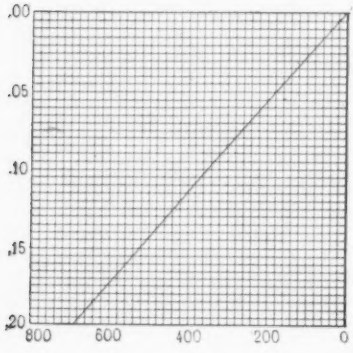

FIGURE 4. — Curve four from Hamburger p 83.

The question arises as to a means of obtaining the inorganic freezing point from conductivity of serum. In G, of Tables X and XII, is given the total percentage of salts in terms of sodium chloride, the freezing point of which is 0.519 and the conductivity at 5° C. is 90. Both of these values are greater than those for the inorganic salts of normal serum. The value of 82 for the average conductivity of normal serum gives a freezing point in terms of sodium chloride of 0.455, which value is too small. The correct freezing point of 0.490, however, is obtained from a sodium chloride solution having a conductivity of 88. This put in other words means that if the normal conductivity of serum be increased by 7 per cent its value in terms of the freezing point of sodium chloride will correspond to the actual inorganic freezing point of serum.

Thus by increasing the conductivity of serum 7 per cent and using Curves 1 and 4 the inorganic freezing point of any serum from a healthy individual may be approximately found.

The facts stated in the last few paragraphs will be made clearer by the following abstract:

Symbol.	Substances.	$\lambda_5 \times 10^5$	$\triangle$
A	0.88% NaCl (from G, Tables X and XII).	90.0	0.519
B	0.131 gram equivalent of NaCl from curves 1 and 4.	82.0	0.455
C	0.88% salts and gases from Tables X and XII.	98.5	0.503
D	(C) when reduced 2% proportionally throughout.	96.6	0.493
E	Normal serum with corrected λ_5 (see column 4, Table VIII).	96.2	0.493
F	Reduced value of salts in Tables X and XII when the λ_5 is depressed by 7.5 grams per 100 c.c.	82.0	0.493
G	Inorganic salts of normal serum.	82.0	0.493

Results obtained from clinical cases. — Table XIII gives results on blood obtained chiefly from patients in the Cook County Hospital. The diseases are arranged in groups to facilitate comparisons and deductions thereon. The columns are arranged as in Table VIII; the gram-equivalents were omitted as they were not essential. The symbol D, column 10, indicates the difference between the value in columns 9 and 8. Where blanks occur either no determinations were made or the results were considered faulty.

Before going on to discuss the separated groups it is well to consider generally some factors in the tables.

When the value of λ_b (conductivity of defibrinated blood) is high it means one or more of three things: an excessive amount of inorganic salts; a diminution in the number of corpuscles; or the increased permeability of the corpuscles as occurs in all the stages of laking. When the λ_b is low the weight of salts must be low or the per cent of corpuscles high. In other words, λ_b varies directly with the amount of salts and the degree of laking and inversely as the percentage of the corpuscles.

A high value for λ_s signifies that the salts must be high or rarely that the proteids must be greatly decreased. Expressed generally λ_s varies directly with the salts and inversely with the non-electrolytes, especially the proteids.

The values of p and h will throw much light on the interpretation of the values gotten for λ_b and λ_s.

The value of $p-h$ is almost 6 per cent in blood from individuals in health but has a wide range in deceased conditions.

A high $\triangle$ means one or both of two things. First a high percent-

TABLE XIII.

1	2	3	4	5	6	7	8	9	10
Number of blood.	Nature of disease.	$\lambda_5 \times 10^5$ at 5° C.	$\lambda_0 \times 10^5$ at 5° C.	Percentage of serum by formula $p = \frac{\lambda_0}{\lambda_5}(174.5-\lambda_5)$.	Per cent by hæmatocrite $= h$.	Difference between p and h.	△ from corrected sodium chloride λ_5.	△ got by Beckmann's thermometer.	9 − 8 due chiefly to non-electrolytes.
Nephritis.									
1	Acute.	30.1	91.1	48.9	48.0	0.9	0.555	0.624	0.069
2	"	43.0	88.8	61.0	57.0	4.0	0.522	0.603	0.079
3	Chronic Parenchymatous Nephritis.	41.8	86.7	63.8	59.2	4.6	0.516	0.597	0.081
4	" "	56.7	90.1	70.1	....	....	0.533	0.661	0.132
5	Delirium Tremens and Nephritis.	31.2	76.0	58.5	52.3	6.2	0.450	0.543	0.093
6	Parenchymatous Nephritis.	34.9	69.4	70.1	56.0	14.1	0.406	0.541	0.135
7	" "	31.4	71.7	62.2	57.9	4.3	0.416	....	....
8	" "	31.2	82.3	54.1	50.0	4.1	0.486	0.602	0.116
9	" "	34.2	83.3	57.6	52.2	5.4	0.492	0.557	0.065
10	Uræmia.	58.0	95.1	72.7	67.0	5.7	0.567	0.803	0.236
Anæmias.									
11	Pernicious Anæmia.	42.2	87.2	66.4	68.4	−4.0	0.516	0.602	0.096
12	Leukæmia A.	45.7	58.4	97.8	88.4	17.4	0.334	....	....

13	Leukæmia B.	45.2	61.7	94.5	80.4	14.1	0.355	....	....
14	Leukæmia.	69.8	105.6	69.8	78.2	−9.6	0.599	....	....
15	Pernicious Anæmia.	86.8	89.8	87.0	95.0	−8.0	0.525	....	....
16	" "	63.7	87.4	77.7	72.2	5.5	0.516	....	....
17	" "	47.9	83.4	72.6	64.4	8.2	0.486	....	....
				SYPHILIS.					
18	Secondary.	33.1	77.9	59.3	50.0	9.3	0.450	0.549	0.099
19	Tertiary.	38.4	77.8	66.2	60.4	5.8	0.456	0.581	0.125
20	"	46.5	98.9	60.2	54.8	5.4	0.587	0.609	0.022
21	Primary.	39.9	82.7	60.7	52.2	8.5	0.516	0.580	0.064
				DIABETES.					
22	Diabetes.	39.0	81.1	65.0	62.0	3.0	0.473	0.592	0.119
23	"	31.8	67.6	66.7	56.3	10.4	0.396	0.560	0.134
24	"	22.2	75.3	52.6	46.3	6.3	0.444	0.614	0.170
25 A	"	29.8	73.3	58.8	50.7	8.1	0.424	0.553	0.127
25 B	Results from second examination of 25 A.	29.8	73.8	58.5	50.7	7.8	0.424	0.549	0.125
26 A	" "	30.8	75.1	58.8	52.2	6.6	0.444	0.570	0.126
26 B	Two weeks after A.	30.2	71.1	61.6	52.2	9.4	0.411	0.545	0.136

TABLE XIII — *continued.*

1	2	3	4	5	6	7	8	9	10
Number of blood.	Nature of diseases.	$\lambda_5 \times 10^5$ at 5° C.	$\lambda_c \times 10^5$ at 5° C.	Percentage of serum by formula $p = \frac{\lambda_5}{\lambda_c}(174.5 - \lambda_5)$.	Per cent by hæmatocrite $= h$.	Difference between p and h.	$-\triangle$ from corrected sodium chloride λ_5.	$\triangle$ got by Beckmann's thermometer.	9 − 8 due chiefly to non-electrolytes.
Pneumonia.									
27	Acute.	28.3	65.3	63.0	55.1	8.2	0.381	0.644	0.263
28	Before crisis.	26.1	75.3	51.2	50.0	1.2	0.438	0.581	0.143
29	" "	30.3	78.3	54.6	54.0	0.6	0.461	0.653	0.192
30	" "	30.4	73.1	58.1	52.3	5.8	0.424	0.597	0.173
Tuberculosis (Pulmonary).									
33 A	Chronic.	31.8	81.1	55.7	52.2	3.5	0.480	0.556	0.076
33 B	"	36.8	72.2	66.3	57.8	8.5	0.416	0.543	0.127
33 C	"	31.2	69.6	65.9	54.4	1.5	0.396	0.574	0.178
34	"	38.7	80.6	65.1	56.5	8.6	0.473	0.568	0.095

	TABES DORSALIS.								
35	Tabes Dorsalis.	37.2	79.6	64.0	52.0	12.0	0.667	0.581	0.114
36	" "	54.9	88.0	75.6	51.6	24.0	0.522	0.609	0.087
	RHEUMATISM.								
37	Fever.	60.0	83.0	81.7	....	....	0.492	....	....
38 A	Chronic.	56.3	96.8	70.0	60.0	10.0	0.573	....	....
38 B	"	66.9	96.2	69.0	61.0	8.0	0.573	....	....
39	Chronic and Anæmia.	57.1	85.9	82.3	65.2	17.1	0.511	0.625	0.114
	MISCELLANEOUS CASES.								
40 A	Pregnancy.	43.5	91.1	62.9	57.7	4.8	0.564	0.596	0.052
40 B	"	44.0	90.1	63.0	57.7	5.3	0.539	0.590	0.051
41	Typhoid.	38.0	84.4	61.6	55.5	6.1	0.499	0.565	0.066
42	Delirium Tremens.	28.5	66.2	58.6	53.2	5.4	0.391	0.643	0.252
43	Pyæmia.	33.6	78.2	59.1	56.7	4.4	0.467	0.563	0.069
44	Abscess and Anæmia.	33.4	90.7	50.7	50.2	0.5	0.533	0.609	0.076
45	Piles.	31.7	76.2	59.7	57.0	2.7	0.450	0.561	0.111

age of salts, or second, an increased weight of organic bodies, such as urea, fat, sugar. If $\triangle$ is low it will be due to a decreased quantity of inorganic elements of inorganic bodies.

The value of D (the difference between $\triangle$ by Beckmann and the calculated inorganic $\triangle$), column 10 will largely decide the amount of the non-electrolytes present. It will vary directly with the non-electrolytes present in serum.

In looking over Table XIII as a whole and contrasting the results with those of Table VIII, one notices that in the majority of cases there is a departure from the normal in regard to one or more of the factors λ_b, λ_s, p, h, $\triangle$, and D.

The first disease in the table is nephritis, of which there are ten cases. Numbers 2, 3, 4, and 10 show a high conductivity for blood and serum, and numbers 2, 3, and 10 also a high p and h, so that we know that the percentage of corpuscles is low and that the salts are increased. The cryoscopic values are also in harmony with this statement. Such patients would probably show a high blood pressure from the high osmotic pressure and have all the other concomitant symptoms.

The anæmias all show high conductivity for blood and one of them, No. 14, a high λ_s. They all show high p and h. The freezing point values with one exception are not recorded, as at the time the specimens were examined I had not yet developed the method for taking freezing point of small quantities.

From the values given for conductivity of the blood one sees the abnormality of the blood.

In connection with numbers 12 A and 12 B an interesting experiment was made; 3 c.c. of blood was obtained from a patient in the last stages of spleno-medullary leukæmia; 1.5 c.c. was put in each of two tubes and sealed. The one 12 B was treated for thirty minutes by X-rays, the other untreated. Both tubes were put on ice. Unfortunately owing to circumstances I was unable to examine them until four days later.

The results, however, gave a hint that the X-rays had modified the permeability of the corpuscles. My theory was that since X-ray applied locally on patients suffering from this disease produces a cytolytic action, that the action might take place under X-ray treatment of the blood in vitro. It was shown by Dr. Capps of Rush Medical College that no microscopic changes are evident in the leucocytes after a half-hour exposure of leukæmic blood in vitro, but

this did not show that the corpuscles had not undergone a change in the permeability of their membrane or in other words altered the conductivity of the blood and serum.

Should a change occur a means might be had for making a cytolytic serum for leukæmia.

I regret that since that time I have not been able to procure sufficient leukæmic blood for the experiment.

TABLE XIV.

1	2	3	4	5	6
No.	Nature of fluid.	Conductivity at 5° C. × 10^6.	△ according to sodium chloride equivalent.	△ gotten by experiment.	Difference between values in columns 5 and 4 = D
45	Syrupy fluid from gonorrhœal knee joint.	80.0	0.473	0.449	− 0.024
46 A	Serum from dog's blood heated to 56 for 30 minutes.	94.2	....	....	....
46 B	Serum from same dog's blood not heated at all.	94.2	....	....	....
47	Fluid from enlarged gall bladder.	86.1	0.511	0.611	+ 0.100
48	Fluid from tuberculous knee joint.	80.8	0.473	0.556	+ 0.083
49	Syphilitic fluid from enlarged knee joint.	77.1	0.450	0.668	+ 0.218
50	Fluid gall bladder (purulent).	88.5	0.522	0.527	+ 0.005

From the syphilis group no general conclusions can be drawn. With regard to five cases of diabetes and four cases of pneumonia we find that the conductivity of blood and serum are low and D high; this indicates that in both these diseases there is an excess of organic bodies. There is one important difference between the two. Diabetes has about a normal △, whereas pneumonia has an increased △. In the diabetic cases the increased amount of sugar of 0.2 per cent [1] will only account for an extra 0.020 in the freezing point; the rest must come from some other non-electrolyte, probably fat.

The other groups do not warrant general deductions except in so far as they furnish some knowledge as to the conditions of the blood.

In the last table, No. XIV, an examination is made of six fluids. The conductivity at 5° C. of the three knee-joint cases is below that

[1] OSLER: Principles and practice of medicine, 1905, p. 412.

of normal serum, but in the other cases the conductivity is greater than that of serum. The fluids from the enlarged gall bladder and swollen knee joint give a lower freezing point as compared to that of serum. The freezing point in this gall bladder case is, however, much below that of normal bile. The average freezing point from four examinations of bile from healthy individuals is given by Stewart[1] as 0.803; hence it can be safely inferred that the swollen gall bladder in case 47 was not due to simple osmosis.

In case 46 A, dog's blood was heated for thirty minutes to 56° C. and 46 B was not heated. No change was detected in the conductivity of the heated serum.

XI. Summary.

1. The electrical method of freezing small quantities is adapted for a physical laboratory but not for clinical purposes.

2. The freezing point of 0.3 c.c. of salt solution can be determined with a fair degree of accuracy when mercury is added to make the volume sufficient to cover the greater part of the Beckmann bulb. The inoculation method must be used in all determinations with small quantities.

3. When the volume of liquid reaches 0.8 c.c. the mercury may be discarded and determinations made without it.

4. Blood serum and blood cannot be mixed with mercury in Δ determinations, since an emulsion so readily forms and spoils the result.

5. Not less than 0.8 c.c. of blood or serum can be accurately frozen for the reason given above.

6. The composition of the organic bodies and inorganic salts of the serum, as given in Tables X, XI, XII, harmonizes with six facts about serum:

A. It represents closely the total weight of the formed bodies in serum.

B. It represents the total alkalinity so far as this can be accurately shown.

C. It accounts almost exactly for the five chief elements found in the salts of serum.

D. It gives the right percentage of sodium chloride.

[1] Stewart: Manual of physiology, 1905, p. 315.

E. When the percentages of the salts are reduced 2 per cent it gives the proper freezing point, *i. e.*, 0.566° C.

F. It gives the proper conductivity for normal serum, *i. e.*, 82.

7. The improved micagocrite has been shown to give reliable results, as seen especially from Table V.

8. Conductivity measurements are best made at 18° C.

9. The micagocrite tube can be used as an ordinary hæmatocrite tube.

10. The freezing point for gases in solution can be got from the equation $\triangle = 8\ x\ (10)^{-5}$ where $\triangle$ equals thousandths of a degree centigrade and x the number of c.c.'s of gas dissolved in a litre of solution.

11. The freezing point equivalent for conductivity of serum, increased 7 per cent given in terms of sodium chloride, gives approximately the inorganic freezing point of serum from healthy individuals. Its value is 0.490° C.

12. The curves plotted are of clinical value in correlating $\triangle$ and λ_5 in terms of sodium chloride. By knowing the freezing point of a solution, the gram-equivalent of an isotonic salt solution can be quickly ascertained, also its value in grams per 100 c.c. by multiplying the gram-equivalent by 5.85. When the conductivity at 5° C. of a solution is known, its gram-equivalent and gram per 100 c.c. can be likewise correlated to a sodium chloride solution.

13. In cases of nephritis, pneumonia, diabetes, and anæmias, information is obtained from conductivity and freezing determinations which appears to be of considerable diagnostic value.

14. In other diseases, considerable knowledge is obtained which may help in the proper understanding of blood conditions. It will, however, remain for future investigators to examine each specific disease in regard to the ultimate value of the above factors.

In conclusion I must thank the internes of Cook County Hospital, and especially Drs. Mathews and Spooner for their courtesies extended to me, so that I might have material for the later part of this research, and finally to my esteemed professor, Dr. G. N. Stewart, who suggested to me this research.

INTESTINAL GASES OF MAN.

By J. AUGUST FRIES.

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FROM numerous investigations by various men the conclusion has been drawn that in every healthy individual there is always present in the digestive tract large numbers of micro-organisms. Some physiologists claim, further, that the presence of certain kinds of putrifying as well as other kinds of bacteria are absolutely necessary for continuous good health, normal digestion, and for the best use of the food, and that as soon as the conditions for the proper development and activity of these micro-organisms are made unfavorable, more or less serious disturbances will be the consequence of it. The special beneficial action of these bacteria is ascribed to certain enzymes produced by them. Bacterial activity is also accompanied by the formation of gas, which is frequently of a combustible nature.

That combustible gases pass daily, and in varying quantities, from a person the writer has long been aware of, but as to the quantity and composition of the same there appear to be but few, if any, definite and satisfactory figures obtainable.

Apparently more is known about the gas production in cattle. In the digestive tract of the herbivora, especially the ruminants, under normal conditions there is always a considerable fermentation of the carbohydrates of the feed, resulting in the formation of combustible gases which escape from the animal body. This process is so constant that it can neither be counted accidental nor incidental, but ought rather to be looked upon as a normal condition, a kind of symbiosis between man and the lower orders of life, as a part of the digestive plan itself. The animal nutrition experiments carried on under the direction of Dr. H. P. Armsby at State College, Pa., by the aid of the respiration-calorimeter, have demonstrated that the quantity of this gas mixture is considerable, the carbon of the same amounting, in the case of a grown-up steer, from about 6 to 8 per cent of the total

carbon metabolized, and its energy represents from 10 to 14 per cent of the total metabolizable energy of the feed.

The composition of the combustible portion of the gases escaping from a steer fed clover or timothy hay, mainly, proved to be that of methane. See pages 31–33, 52, U. S. Dept. of Agriculture, Bureau of Animal Industry, Bulletin 51, and Bulletin 74, pages 18, 19, 34, 35.

The daily production of methane in cattle is really enormous, its volume (other gases not included) ranging from 100 to 250 litres and more as the feed is increased. In nutrition experiments with cattle, therefore, the gas formation must always be taken into consideration.

In the nutrition experiments with human beings the intestinal gases appear to have been left out of account entirely, and investigators who are engaged in this line of work, though being well aware of the fact of gas formation, consider, or rather assume, the quantity of combustible gases formed to be too small to introduce any appreciable error in the results of the experiments. This latter depends, of course, entirely upon as to what degree of accuracy one expects to work. Such an assumption as just referred to may be partly justified because of certain facts. For instance, the human intestine is very much shorter than that of cattle, — the intestine of the latter being about twenty times the length of the body, while in man it is perhaps not more than ten times longer than the body. In cattle we find four stomachs, but in man only one. Man, as a rule, eats a mixed ration of meat and vegetable matter, while cattle eat plant food, which consists largely of carbohydrates, and the feed may remain in the body of the ox several times as long as food does in the case of man. The kind of food we eat, and the short time of its detention in the body, are factors which would, naturally, make the conditions for much gas formation less favorable than they are in the ruminants.

Combustible gases, however, do escape from man, and the writer has long wished to satisfy himself upon the point of how large an experimental error they represent and to get some definite figures as to quantity and composition of the gases. During the past winter, therefore, I took the opportunity to carry on an investigation with myself for the purpose stated, and the following will give a brief description of the work and its results.

Physical condition and food. — The writer was, at the time of the investigation, in perfect health, including good digestion. Body weight, about 140 pounds. The daily fare was very plain, and barely

a maintenance quantity was consumed. The food was not weighed, but the common expression, "not a big eater," will perhaps signify as much as a few weight figures would. No alcohol, and practically no spices, were used. The bill of fare for one day, which may serve as an example, was: breakfast — oatmeal, Graham bread, butter, and coffee; dinner — soup and crackers, roast beef, potatoes, boiled rice and milk, Graham bread, butter, and coffee; supper — a small piece of beefsteak, stewed potatoes, bread, butter, and cocoa. During the day there was no heavy muscular work performed, but considerable walking about, in connection with the work engaged in.

Collection of gas sample. — A simple apparatus was constructed by means of which the rectal gas discharges could be collected with only a minimal introduction of outside air. From this apparatus the gas was transferred to a flask, by displacement of water, and kept over water until it was to be analyzed. A portion of the first lot of gases collected for analysis had been allowed to stand for a number of hours in thin rubber receptacles, and it was noticed that a quantity of gases had diffused through the rubber. The gases were, nevertheless, after the carbon dioxide and water had been removed from the same, oxidized by means of platinized kaolin heated to redness in a copper combustion tube, and the resulting carbon dioxide and water collected by the use of soda-lime and sulphuric acid and weighed. For further description of the apparatus see Experiment Station Record, Vol. XV, No. 11, p. 1042; also Proceedings Society Promotion of Agricultural Science, 1902, p. 111.

Analytical results. — The total sample of gas at 0° and 760 mm. was 1254 c.c., and the result of the analysis was

CO_2 as such in the gas	0.0571 gm.
CO_2 formed by combustion	0.4302 "
H_2O formed by combustion	0.3555 "

Assuming that all the combustible carbon was united with only hydrogen and present as marsh gas, we would have by volume

CO_2 =	28.6 c.c.
CH_4 =	201.0 "
H_2 =	35.7 "

On account of the loss of some of the gases by diffusion through rubber, the results of this first lot of gases will not be used in the final calculation.

While collecting a second lot of gases for the carbon and hydrogen determination, the separate charges were guarded against loss through diffusion as much as possible. This lot was also collected and kept over water, but was not washed in as much water as was the first lot of gases. A small quantity of the carbon dioxide must of necessity have been removed by the water, and hence the carbonic acid found in this sample does not represent the actual quantity present in the gas mixture. After correcting for the unavoidable but small quantity of air introduced, the volume of the gas sample, reduced to 0 and 760 mm. pressure, was 1814 c.c. This gas was passed over pumice-stone saturated with sulphuric acid and soda-lime for the removal of the water and carbon dioxide present, and then through the combustion tube referred to above for the oxidation of combustible gases. The results of the analysis were:

CO_2 as such in the gas sample = 0.2807 gm.
CO_2 after combustion = 1.1794 "
H_2O after combustion = 0.9602 "

The water found is a little less than what corresponds to the carbon dioxide to form marsh gas of the carbon and hydrogen. The difference, however, is not large, and it is barely possible that in this case, where such a large quantity of gas was oxidized rather quickly, the last traces of moisture condensed in the tubes between the combustion tube and absorption apparatus were not removed completely by the amount of ventilation which ordinarily is sufficient for this apparatus. Since in the previous lot or sample a little excess of hydrogen was noticed, we may in this case, without introducing any appreciable error, reckon from the carbon dioxide to methane. We then have:

1.1794 gm. CO_2 = 0.4297 gm. CH_4 = 597.6 c.c. CH_4.

In order to get the total carbon dioxide present as such in the gas, a sample was collected over mercury, and the carbon dioxide absorbed by means of potassium hydrate. The corrected volume of the gas sample was:

Before absorption	175.6 c.c.
After absorption	157.2 c.c.
Difference	18.4 c.c. = 10.48% CO_2 by volume.

In the same sample oxygen was determined by the use of pyrogallol and potassium hydrate solution, but only a trace of oxygen was found.

The action of the pyrogallol was questioned, and another sample was collected over water, and without coming in contact with much water it was analyzed for carbon dioxide, oxygen, and carbon monoxide, phosphorus being used for the oxygen determination. The following were the results obtained:

$CO_2 = 10.1\%$ by volume
$O_2 = 1.3$ " "
$CO = 0.0$ " "

Basing our calculations upon these last three samples, which were taken at different periods and analyzed, and taking the average of the figures obtained for carbon dioxide and oxygen, the marsh gas as reckoned from the combustible carbon, and calling the remainder free nitrogen, the following will be the composition of the rectal discharges of intestinal gases of man:

$CO_2 = 10.3\%$ by volume.
$O_2 = 0.7$ " "
$CH_4 = 29.6$ " "
$N = 59.4$ " "

That the combustible portion of the gases consists of marsh gas speaks further the facts that the gases, collected as they were over water, were odorless and burned with but a slightly luminous flame.

The large quantity of free nitrogen the writer does not claim to be a decomposition product, but rather believes it to be atmospheric nitrogen swallowed as air with the liquid and solid foods and with saliva, the oxygen having been absorbed into the blood, or has to some extent served to supply living organisms in the alimentary canal with oxygen. Should a small portion of this nitrogen come from decomposed nitrogenous substances, there is no way of determining it directly.

Daily gas production. — The single discharges of gas vary in volume a great deal, but the daily total appears to be much less variable. Thus the single discharges ranged from 50 c.c. to nearly 500 c.c. (the latter after several hours' withholding of the discharge), the average being somewhat more than 100 c.c. The actual quantity of gas collected a day was one day nearly 700 c.c., and with the estimated amount not collected, the production for the day would be over 900 c.c. On another day about 600 c.c. was collected, and with the estimated loss the total for that day would be between 800 and

900 c.c. On other days the indications were that the production would exceed the quantities already mentioned, so that in round figures for a healthy, medium-sized man on very plain food such as mentioned before, 1 litre of practically odorless intestinal gases a day as rectal discharge can be considered a none too high average. To what extent individuals will differ in this respect, or what the effect would be of a rich meat diet of highly nitrogenous plant food such as legumes, of disturbances in the digestion process, or other ailments, upon the formation of gases is not known.

Magnitude of experimental error due to intestinal gases. — According to the composition given 1 litre gas would contain 296 c.c. equal to 0.2128 gm. marsh gas. This quantity methane represents 2.84 large calories, 1 gm. marsh gas being reckoned equal to 13.344 small calories. A man at light muscular work will, according to Tigerstedt use 42 calories per kilogram body weight, hence a man weighing 63 kgs. would under such conditions use about 2650 large calories a day. Assuming that the ratio of carbon dioxide to heat produced is 35 gms. carbon dioxide to 100 large calories, the daily metabolized carbon would be 253 gms. In 0.2128 gm. marsh gas there is 0.1593 gm. carbon.

The experimental error in nutrition investigations due to this gas would be

$$\text{For energy } 2.84 \div 2650 = 0.11\%$$
$$\text{For carbon } 0.1593 \div 253 = 0.06\%$$

The writer believes, judging from common observations only, that in the case of cattle the larger portion of the fermentation gases leave the body through the mouth and nose. As to what extent the fermentation products escape through the nose and mouth in the case of man I am not prepared to state at this time, but certain it is that many persons having a more or less severe attack of indigestion will expel gases from the stomach through the mouth. Persons with the so-called " wind in the stomach " will in a few hours expel more gases by way of the mouth, by the process of eructation, than the daily rectal discharge amounts to, but the exact quantity, composition, or the error which such gases would represent in a metabolism experiment, have not been determined.

As to the significance of these gases, of this particular composition, or what rôle they play in the body, if they have any, we are entirely ignorant. Why should provision be made to have practically all the

oxygen removed, leaving a mixture of the three indifferent gases,— carbon dioxide, marsh gas, and nitrogen? We can but conjecture and ascribe to it a rôle of usefulness in the scheme of digestion and assimilation of the food, in that this gas mixture may serve as a regulating agency to regulate the growth of micro-organisms in the digestive tract, checking the growth of some, preventing the growth of others which may find their way into the intestines.

THE EFFECT OF CHANGES IN BLOOD-PRESSURE ON RESPIRATORY MOVEMENTS.

By C. C. GUTHRIE and F. H. PIKE.

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IN the course of some experiments on the effect of changes of blood-pressure on certain mammalian physiological mechanisms [1] the question arose as to the effect of changes of blood-pressure upon respiration. The present paper embodies the results of experiments on respiratory movements.

Review of the Literature.

Stenson [2] and Swammerdam [3] in the latter half of the seventeenth century, first ligated the aorta of animals, thereby producing a high blood-pressure in the anterior part. Although this experiment is named after Stenson, who ligated the aorta of fishes, it should, as Fredericq [4] points out, be called Swammerdam's experiment in mammals as he first ligated the aorta of a mammal. It will readily be seen that temporary ligation of the abdominal or thoracic aorta gives a means of studying changes in the respiratory movements following changes in blood-pressure without the use of drugs other than the anæsthetic.

Swammerdam's original paper is not accessible to us. Subsequent investigators have either omitted or incompletely reported observations on the respiration in repeating these experiments. Extensive reviews of the literature on this point are given by Colson [5] and Rothmann.[6]

[1] Guthrie and Pike: Science, 1906, xxiv, p. 52. Preliminary note.

[2] Stennonis, Nicolai: Elementorum myologiae specimen, Florence, 1667.

[3] Swammerdami, Johannis: Tractatus de respiratione, Ludwig, Batavia, 1667.

[4] Fredericq: Archives de biologie, 1890, x, p. 131.

[5] Colson: Archives de biologie, 1890, x, p. 431.

[6] Rothmann: Archiv für Physiologie, 1899, p. 120: *Idem*, 1900, p. 365; Neurologisches Centralblatt, 1899, pp. 2, 61: *Idem*, 1900, pp. 16, 66.

Very few positive statements of the effect on respiration of changes of blood-pressure have been found. Starling[1] says that "Well-marked Cheyne-Stokes breathing has often been observed in cases where there was increased cerebral pressure or deficient blood supply to the brain. We[2] have considered in a previous paper the changes in the respiratory movements caused by cerebral anæmia. Bohr[3] found that the reduction in carbon dioxide elimination was greater after injections of peptone than after injections of leech extract, the ratio $\frac{CO_2}{O_2}$ being 0.38 to 0.77 for peptone, and 0.60 to 1.01 for leech extract. Whether this decreased elimination was due to the peptone itself or to the low blood-pressure caused by the peptone cannot be decided by Bohr's experiment. Nothing is said concerning changes in the respiratory movements. Pembrey and Gürber[4] making experiments in which severe bleeding caused syncope, dyspnœa, or even cessation of respiratory movements, found that changes in the respiratory quotient $\left(\frac{CO_2}{O_2}\right)$ following bleeding with subsequent transfusion were not marked (rabbits). Loss of blood to the extent of two per cent of body weight without subsequent transfusion was followed by a slight increase in the respiratory exchange. The relation of these phenomena to blood-pressure is again problematical.

Hill[5] believes that a certain blood-pressure is necessary to provoke respiration, and that when Traube-Hering curves, due to anæmia, morphia, or magnesium sulphate, appear in the blood-pressure tracing, the Cheyne-Stokes respiration bears a certain relation to them, the respiratory centre being stimulated to greater effort by each rise of blood-pressure.

Experimental Results.

Healthy cats were used. The animals were etherized and tracheotomy performed. Respiratory traces were taken by means of a tambour connected with the tracheal cannula, the excursion of the

[1] Starling: Schaefer's Text-book, 1898, ii, p. 309.

[2] Stewart, Guthrie, Burns and Pike: Journal of experimental medicine, 1906, viii, p. 289.

[3] Bohr: Centralblatt für Physiologie, 1888, ii, p. 261.

[4] Pembrey and Gürber: Journal of physiology, 1893, xv, p. 449.

[5] Hill: The Cerebral Circulation, 1896, p. 132. Philosophical transactions of the Royal Society, 1900, 113 B, pp. 77-85.

writing point being regulated by a screw clamp on a side tube. Blood-pressure tracings were taken from the central end of the carotid artery. With the animal lying on its side an incision was made along the dorsal vertebræ and the skin reflected for a short distance laterally. A large aneurism needle was passed through the chest wall on one side a little in front of the vertebral column, and the point brought out on the other side. A ligature was then drawn through and the needle removed. Traction on the ligature caused a rise in blood-pressure by occluding the aorta, with a fall when it was released. Variations in blood-pressure of 100 per cent may be produced in a few seconds. No effect is produced by tightening the ligature when it is passed behind the aorta. The most favorable place for operation is from the eighth to the tenth ribs.

Dogs were used for comparison. In the first experiment to make sure that incision of the thoracic muscles did not itself affect the blood-pressure and respiratory movements, the abdomen was opened, a ligature passed around the aorta, and the ends brought out through a glass tube of sufficient length to have one end outside of the abdominal cavity when the other rested upon the aorta. The wound was then closed with stitches. Traction on the ligature occluded the aorta by pulling it up against the end of the glass tube, which was firmly held in position. Later, an aneurism needle was passed through the chest wall and the thoracic aorta occluded, as in cats. Occlusion of the thoracic aorta of course gives greater blood-pressure changes than occlusion of the abdominal aorta, but there are no qualitative differences. Therefore in the other experiments on dogs the thoracic aorta was at once occluded.

The blood-pressure changes, together with the changes in respiration, are given in the accompanying tables.

Discussion of Results.

A sudden rise of blood-pressure with vagi intact is, as a rule, followed by an increase in the respiratory rate. This is more particularly true for the first rise, and for subsequent rises which occur after comparatively long periods of normal pressure. Thus with a normal blood-pressure of 144 mm. of mercury, we have 24 respirations in twenty seconds; with a rise of pressure to 196 mm. the rate becomes 28 in twenty seconds. Again, an increase in pressure from 124 mm. to 168 mm. of mercury is accompanied by an increase from 20 to 26 res-

pirations in twenty seconds. Sustained high pressure is accompanied by a uniform or by an increased rate. A pressure of 178 mm. gave a rate of 20 in the first twenty seconds and 24 in the next twenty seconds. A sudden fall in pressure is generally followed at first by an increase in rate or by no change. Usually also, there is later a period

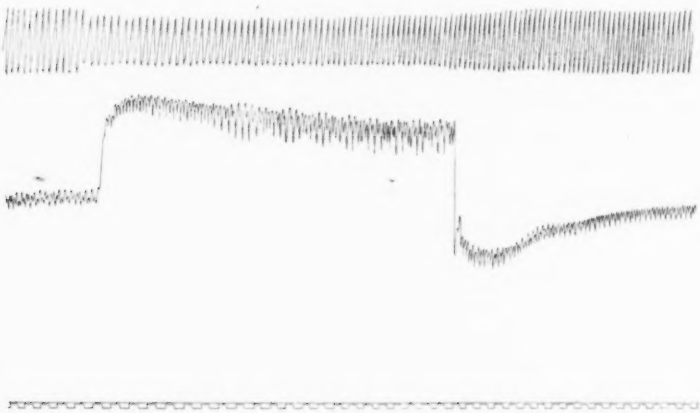

FIGURE I. — Four-ninths the original size. Dog. Respiration at top, with blood-pressure just below. Vagi intact. Time in seconds. Base line immediately above time. Blood-pressure and respiration for successive periods of 20 seconds were: 140 mm., 24 respirations; 200 mm., 27 respirations; 188 mm., 28 respirations; 114 mm., 29 respirations; 135 mm., 29 respirations.

of slowing. Thus, following a pressure of 185 mm. with a rate of 29 in twenty seconds, a pressure of 110 mm. is associated with a rate of 27 in the first twenty seconds after the fall, and the rate of 22 in the next twenty seconds. Again, a fall from a long sustained pressure of 178 mm., with a rate of 24 in twenty seconds, to a pressure of 115 mm., gave a rate of 28 in the first twenty seconds, and 25 in the next twenty seconds. Occasionally also, there is an immediate slowing after the fall (Table I., Fig. 1).

Apparently anomalous results appear in the tables. For example, an increase from a pressure of 118 mm., with a rate of 26 in twenty seconds, to 178 mm. gives a rate of only 22 in twenty seconds. A fall from this latter pressure to 117 mm. gives a rate of 24 in the first twenty seconds, with a subsequent rate of 20 in twenty seconds at a pressure of 125 mm.

After the vagi are divided essentially the same results are obtained (Table II., Fig. 2).

TABLE I.

SHOWING THE RELATION OF CHANGES IN RESPIRATION TO CHANGES OF BLOOD-PRESSURE IN CATS. VAGI INTACT.

Blood-pressure in mm. of Hg.			No. of respirations in 20 seconds.	Blood-pressure in mm. of Hg.			No. of respirations in 20 seconds.
Before occlusion.	During occlusion.	After release.		Before occlusion.	During occlusion.	After release.	
(1) 144	....	....	24	(6) 124	....	....	20
	196	....	28		168	....	26
	185	....	29			118	26
		110	27		176	....	22
		112	22			117	24
(2) 112	....	....	17	(7) 125	....	....	20
	170	....	21		178	....	20
		104	23		178	....	24
(3) 110	....	....	26			115	28
	174	....	25	(8) 127	....	....	25
		110	25		176	....	27
		115	25		178	....	25½
(4) 122*	....	...	26		182	....	23
	144	....	26			90	23
		116	28		174	....	21½
		118	23	(9) 144	....	....	18
(5) 120*	....	....	23		200	....	13
	140	....	25		182	....	13
	140	....	22			102	16
		102	34			100	16
		117	34				

* Dog.

The amplitude or force of the respiration, as indicated by the excursion of the writing point of the tambour, usually is less during high pressure, but shows a *constant* augmentation when the pressure falls.

THE INTERPRETATION OF THE RESULTS.

Two factors, acting in opposite ways, are probably concerned in the establishment of the respiratory rate, and neither must be lost sight of in the interpretation of the results. There is, first, the changing blood-pressure, and, second, the change in the carbon dioxide content of the blood. Bienfait and Hogge[1] have shown that the respiratory rate varies directly with the carbon dioxide content of

[1] BIENFAIT et HOGGE: Archives de biologie, 1890, x, p. 139.

the blood. When the thoracic aorta is ligated, the blood supply to the greater part of the oxygen-consuming tissues of the body is greatly reduced. The posterior part of the body is not completely anæmic, for Colson [1] has shown that, with the thoracic aorta ligated, potassium ferrocyanide injected into the external jugular vein of an

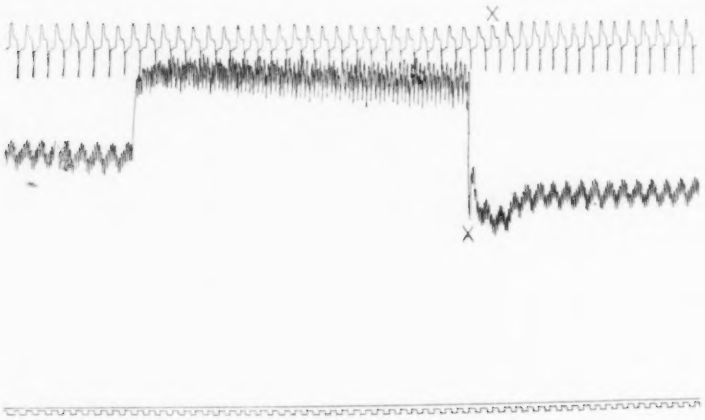

FIGURE 2. — Four-ninths the original size. Dog. Respiration at top, with blood-pressure just below. Vagi divided. Time in seconds. Base line immediately above time. Blood-pressure and respiration for 20 second intervals as follows: 170 mm., 8 respirations; 230 mm., 8 respirations; 230 mm., 8 respirations; 140 mm., 9 respirations; 145 mm., 7½ respirations; 145 mm., 7 respirations.

animal may be demonstrated in the blood of the femoral vein six to nine minutes later. The blood supply must, however, be inadequate to maintain these tissues in their normal state of gaseous exchange. Hence, carbon dioxide must accumulate in them during the period of anæmia and overload the first blood coming from them after the release of the thoracic aorta.

Applying these considerations to the interpretation of the results we must assume that, before the aorta is constricted, all the tissues of the body are normally oxygenated. When the ligature is tightened, the carbon dioxide content of the blood in the anterior part of the body is decreased.

We should expect along with a decrease in the carbon dioxide content of the blood in the anterior end of the animal a decrease in the respiratory rate. This result has actually followed in one ex-

[1] COLSON: *Loc. cit.*

periment, as will be pointed out below. But, instead of a decrease, there is usually an increase of the respiratory rate, due, we must conclude, to the increased blood-pressure. A small rise in pressure may not be sufficient to outweigh or even to balance the diminished carbon dioxide content of the blood and a slower rate may result.

TABLE II.

SHOWING THE RELATION OF CHANGES IN RESPIRATION TO CHANGES OF BLOOD-PRESSURE AFTER DIVISION OF THE VAGI IN CATS.

Blood-pressure in mm. of Hg.			No. of respirations in 20 seconds.	Blood-pressure in mm. of Hg.			No. of respirations in 20 seconds.
Before occlusion.	After occlusion.	After release.		Before occlusion.	After occlusion.	After release.	
(1) 143	....	....	13		174	....	10
	196	....	13		174	....	15
		132	13			118	16
		132	13			128	12
(2) 146	....	....	11½	(7) 134	....	....	10
	196	....	13		190	....	7
		123	14			100	8½
	196	....	15½	(8) 134	....	....	7
		112	11		200	....	5
(3) 120	....	....	12			110	7
	204	....	13	(9) 120	....	....	10
	197	....	12		210	....	11
		116	11			82	9
(4) 146	....	....	12	(10) 160	....	....	7
	208	....	14		212	....	13
(5) 132	....	....	12			162	6½
	148	....	11	(11) 160	....	....	6
		100	12		218	....	7
(6) 140	....	....	18				

When the ligature is loosened and the blood again allowed to flow through the posterior part of the body, that which first returns must be surcharged with carbon dioxide. This excess of carbon dioxide tends to cause first of all a quickening of the rate or to prevent a decrease. The normal state of oxygenation of the blood is soon restored and the carbon dioxide content falls to that found before occlusion. The respiratory rate accordingly decreases after a short time.

Considering now the apparently anomalous results, we can readily see that if one period of occlusion and release is quickly followed by a second occlusion of the aorta, the second rise of pressure may not be sufficient to balance the sudden diminution of the carbon dioxide reaching the centre and there may be a decrease in respiratory rate.

We conclude, therefore, that under the conditions of our experiments the usual effect of any considerable increase in blood-pressure is an increase in the respiratory rate with diminished amplitude, while a fall in pressure causes a slower rate with greater amplitude.

Two general explanations of these phenomena appear open to us. First, the increase of blood-pressure may act on the centre directly, as stated by Hill. Secondly, the increased blood-pressure may, perhaps, lead to increased metabolism and greater carbon dioxide output. The increase in the respiratory rate may then be only a secondary effect of the increased metabolism. At present we have no decisive evidence in favor of either view.

The results may afford an explanation of certain clinical phenomena. It has been observed that the sudden withdrawal of ascitic or pleuritic fluid is accompanied by changes in blood-pressure and respiration. These blood-pressure changes are probably sufficient to account for the respiratory changes, although it is possible that irritation of the lungs during the withdrawal of pleuritic fluid may play some part in these changes.

In studying the effect of drugs and tissue extracts on respiration, the changes must be shown to be independent of blood-pressure changes due to the substances used before definite conclusions can be drawn. For instance, the conclusion has been drawn that marrow extract directly stimulates respiration,[1] but this is unwarranted by the evidence presented, although there is some evidence that marrow extract may act on the afferent respiratory nerve endings in the lungs. In the recent work of Doyon and Kareff[2] on atropine and hyoscyamine, the slower and deeper respiration during the period of low blood-pressure may be due to the low pressure and not to the drugs used.

We are indebted to Professor G. N. Stewart for criticism of the proof sheets.

[1] Brown and Guthrie: This journal, 1905, xiv, p. 338.

[2] Doyon and Kareff: Journal de physiologie et pathologie générale, 1906, viii, p. 229.

THE EFFECT OF CHLORALHYDRATE UPON THE PROPERTIES OF HEART-MUSCLE.

By W. H. SCHULTZ.

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RECENTLY there appeared in the "Archiv für experimentelle Pathologie und Pharmakologie" an article by Rohde[1] in which he discusses the effect of chloralhydrate upon the heart of the frog. His conclusions may be given as follows:

1. The refractory period, during early stages of the poisoning, is shortened without any change in the threshold value of the stimulus. In more advanced stages of poisoning the refractory period is apparently abolished.

2. The all or none law ceases to hold.

3. Summation may occur in early stages of the poisoning at a time when the contractile energy of the muscle has in no wise been impaired.

4. With gradually advancing stages of poisoning, the heart, in response to stimuli of a low frequency, yields compound contractions resembling incomplete tetanus, and finally yields a true summated tetanus.

5. Through the agency of chloralhydrate the intrinsic nerve centres of the heart are paralyzed before the fibres are. This is the equivalent of anatomically removing the otherwise inseparable centres of the apex so that under these conditions the heart-muscle acts as a strip of intestinal muscle or as a Limulus heart deprived of its centres.

In view of the significance of these conclusions, should they prove correct, I have repeated Rohde's experiments with results such as will be set forth in this paper.

[1] ROHDE: Archiv für experimentelle Pathologie und Pharmakologie, 1905, lxiv, p. 104.

METHOD.

Three series of experiments were carried on, the first of which included experiments upon the heart, *in situ*, of Pseudemys elegans and P. rugosa and upon ventricular strips taken from the same species. The second series included experiments upon the heart of Rana helecina, and the third series upon the heart of large specimens of Rana cetesbiana.

In the second and third series of experiments the skin was clipped just above the occipital condyles and a narrow-bladed scalpel thrust into the slight depression immediately back of the tip of the parasphenoid until the medulla was reached. Then by carefully inserting a bent ligating needle into the incision and passing it first forward and then backward, the brain and anterior portion of the spinal cord were destroyed with scarcely any loss of blood. The animal was then securely pinned, dorsal side downward, to a flat piece of cork, — the skin, muscle, and sternum severed by a longitudinal incision and spread apart so as to expose the heart. Then the heart was clamped in the region of the auriculo-ventricular junction by means of a Gaskell clamp, yet not firmly enough to cut off the blood from the ventricle. Into the apex of the ventricle was inserted a fine platinum hook which was fastened by means of a thread to one end of a straw-recording lever directly above the heart. This hook also functioned as one of the electrodes. The other electrode was a fine needle so placed that its point could be brought into contact with any portion of the ventricle without materially injuring the surface of the heart or interfering with its movement. Single break shocks from an induction coil were used, the primary coil being supplied by a current from one or two large Edison-Lalande cells. The threshold stimulus was determined as soon as the specimen was in place, and its relative strength was expressed by the distance in centimetres between the primary and secondary coils.[1] Except when testing the absolute refractory period[2] this strength of stimulus was used until a change in the irritability of the heart was noticed, whereupon the strength of the stimulus was increased or decreased so as to insure the use of a stimulus slightly above the threshold. It was necessary to exercise this precaution inasmuch as the hearts of different frogs, and indeed of each individual frog, may vary in irritability.

[1] For convenience the strength of stimulus will be spoken of as 51 cm., etc.

[2] Absolute refractory period corresponds to the time of systole.

Two sets of records were taken in each experiment. The first set included a series taken before the injection as soon as possible after the heart was exposed, whereas the second set included records taken under like conditions except that from time to time measured quantities of chloralhydrate solution were injected into the anterior abdominal vein. This was done with a syringe which could be refilled and manipulated without interfering with the heart, the contractions of which were being recorded without intermission except during the few minutes required to change the kymograph paper.

The moment of stimulation was recorded upon the myogram by means of a spark[1] from an induction coil. This method insures great accuracy, and one is thus enabled to determine in what phase of the contraction the stimulus becomes effective.

Results.

In dosing the heart with chloralhydrate (0.75 per cent), it was found that so large an injection as 4–6 c.c. usually resulted fatally, and that under these conditions successive stages of poisoning followed each other so quickly as to render the study of them difficult. On the whole, the dose which proved most satisfactory for initial injections was 2 c.c., after which doses of such size were administered as would keep the heart under the influence of the drug.

The first effect noticed after the injection of 2 c.c. of chloralhydrate was a marked decrease in the height of the contraction, accompanied either by a partial or temporary loss of rhythmicity. If a like dose were repeated before the heart had to any considerable extent recuperated, the added depression was likely to prove fatal, as was the case with too large an initial dose. However, if sufficient time elapsed between the first and second injections, recovery set in, and the height of the diminished contraction gradually approximated that of the normal contraction. During early stages of the experiment notable changes in the heart phenomena were evident, — the refractory period was shortened, the heart responding during any part of the diastolic phase to such stimuli as were used in determining the original threshold stimulus; appropriate stimuli usually

[1] This method is a modification of the one described by E. A. Schäfer in the Internationale Monatschrift für Anatomie und Physiologie, 1888, v, p. 152. See Fig. 1 for diagram illustrating the principal parts of the key devised for sending in break shocks and recording the moment of stimulation simultaneously. A detailed description of this key will be published later.

became effective at a time corresponding to late systole; the heart upon the application of successive stimuli yielded compound contractions resembling summation and tetanus; the irritability gradually increased to a given maximum, after which it steadily decreased as the poisoning advanced; the power to contract automatically was more or less impaired and the heart on the whole showed a decreased rate of contraction.

During later stages of the poisoning the heart grew more and more sluggish; the phenomena of so-called summation and tetanus became more prominent; the power of the ventricle to yield automatic contractions was lost; as the contractility diminished stronger induction shocks had to be administered in order to call forth extra contractions during a given diastole; and finally the muscle ceased to respond even to very strong shocks.

Refractory Period.

It is well known that electrical stimuli administered during the systolic phase of the normal heart are ineffective, and that a weak stimulus sent in during late diastole which calls forth an extra systole might fail to do so if applied to the heart during early diastole. It would seem, therefore, that the refractory period may be limited to the time of systole plus a portion of diastole. By applying appropriate stimuli the portion extending into diastole may be shortened. This portion of the refractory period is for convenience designated as the *variable refractory period*, whereas that portion corresponding to the time of systole and which towards electrical stimuli remains constant, is called the *absolute refractory period*. To illustrate, in Fig. 2, *a*, dots 4, 6, . . . 24 represent effective stimuli, and 2 represents an ineffective stimulus. In the first contraction the last non-refractory point lies somewhere between *c* and *d*, this point being called *x*. The variable refractory period, then, corresponds to the portion of the cardiac cycle represented by *a b x*. In Fig. 2, *b*, it will be seen that stimuli 4, 8, 10, 12, 14, 16, and 22 are within the absolute refractory period; stimuli 18 and 20 at the end of systole mark the termination of the absolute refractory period, the latter period extending, as above stated, from the beginning of the systolic phase to the last non-refractory point therein. Hence stimuli 4 and 22 of this figure fall within the absolute refractory period, whereas 6 falls within the variable refractory period.

From an example analogous to Rohde's Fig. 1, *a*, *b*, p. 107, it can be seen that the early shortening of the refractory period described

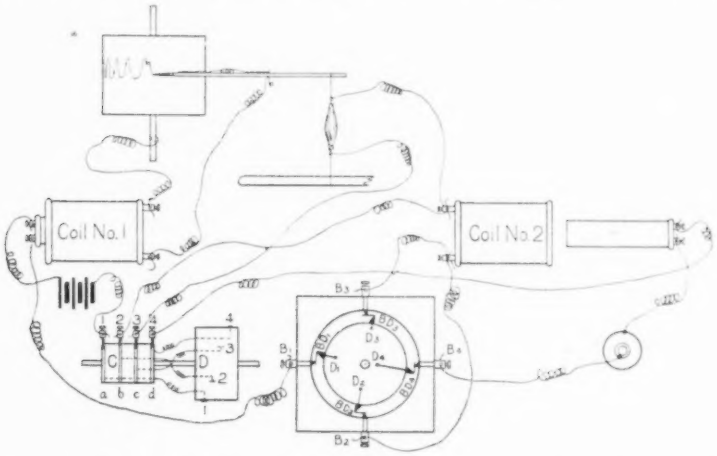

FIGURE 1. — To show the arrangement for recording the moment of stimulation on the cardiogram. By means of a special key a break induction shock is sent into the heart from coil 2 at the same instant as an induction current from a large Rhumkorff coil (coil 1) is sent through the recording lever. The key is composed of two principal parts, *B* and *D*, and *C*. *B* and *D* consists of two parts, the drum *D* with four contacts, *D* 1, 2, 3, and 4; the brush holder with four copper brushes joined to binding posts, *B* 1, 2, 3, and 4 respectively. A cross section of *B* and *D* is shown by the lower right hand figure which not only illustrates the arrangement of the contacts and brushes, but also the method of connecting the contacts to the wires which lead to the bands of the commutator *C*. This commutator forms the second part of the key, serving to continue the circuit which may be closed in the revolution of the drum by any one of the contacts *D* 1, 2, 3, or 4 respectively. This is accomplished by these four copper brushes being connected with binding posts 1, 2, 3, and 4 and being in contact with bands *a*, *b*, *c*, and *d* respectively. Coil 1 generates the spark used in recording the moment of stimulation when the circuit of its primary is broken at BD_1. Coil 2 generates the induction shock used as a stimulus when the circuit to its primary is broken at BD_4, the circuits at BD_4 and BD_1 being broken simultaneously. In order to rule out the make shocks of coil No. 2, the secondary coil is short circuited at BD_2. After the make shock has been short-circuited, contact BD_2 is broken and the muscle is thrown into the circuit of the secondary of coil 2 by making contact BD_3. After this if the drum be rotated so as to break contacts BD_1 and BD_4, the muscle is stimulated at the same time that a spark generated by coil 1 knocks off a bit of soot from the record, thus recording the moment of stimulation.

by him involves only the variable refractory period. Let it be supposed that the atropinized heart yielded contractions like those in Fig. 2, *b*, and that such a heart, though responding to a stimulus in

the diastolic phase at point 32, failed to do so at point 31. After treating the heart with chloralhydrate it responded not only to stimuli 31 and 32, but also to a stimulus of equal strength that was applied at the beginning of the diastolic phase. One effect, therefore, of the chloral poisoning was to shorten the refractory period. The first non-refractory point having been moved from between points 31 and 32 to the end of the systole, it is clear that only the variable refractory period was shortened. As is known, this variable refractory period can be shortened either by increasing the strength of the

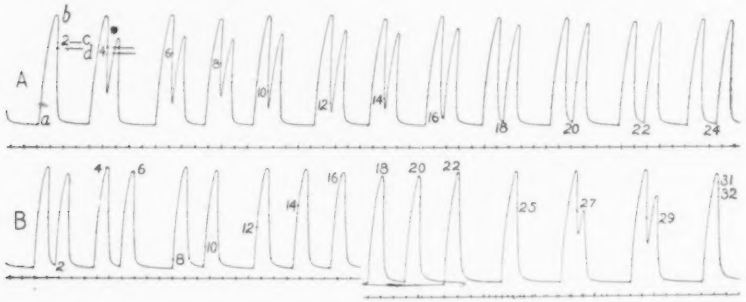

FIGURE 2. — About one-half the original size. *A* and *B* taken from a ventricular strip (*P. elegans*) *A*. — *a b x*, (*x* being the last non-refractory point between *c* and *d*) of the first contraction represents the variable refractory period; points 4, 6, . . . 24 represent effective stimuli in the period of diastole. *B*. — The absolute refractory period corresponds to the time of systole, included between points 18 and 20. Points 4, 8, . . . 16 and 22 are in the absolute refractory period; stimuli 6, 25, 31, and 32 are in the variable refractory period.

stimulus or by increasing the irritability of the heart. My experiments,[1] unlike Rohde's,[2] indicate that an increased irritability accompanies the shortening of the variable refractory period. It is reasonable to suppose that this irritability is a result of the changed condition of the muscle, which in turn causes a shortening of the variable refractory period.

Fig. 3, *f*, is a tracing of Rohde's Fig. 2, *b*. It will be noticed that for the third contraction the fourth to seventh stimuli inclusive fall within the time of systole, and that this contraction is greater in extent than the first, second, and fourth contractions. This increased height

[1] See record of experiments, p. 498.

[2] ROHDE: "Doch habe ich vielfach feststellen können, dass der Schwellenwert der wirksamen Reize in keiner Phase der Chloralvergiftung absinkt." *Loc. cit.*, p. 108.

in the contraction Rohde attributes to the combined action of the aforenamed stimuli. Likewise Rohde leads one to infer that for the fifth contraction, the tenth to thirteenth stimuli inclusive, each acting in its turn, result in a heightened systole like that described for the third contraction. In addition to the apparent action of these successive

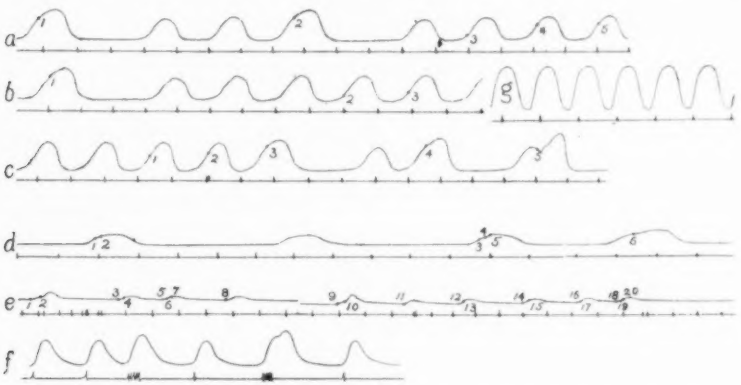

FIGURE 3. — About two-thirds the original size. *a*, *b*, *c*, thirty-four minutes after first intravenous injection. 10 c.c. of 0.75 per cent chloralhydrate solution having been injected in doses of 2 c.c. each. Threshold stimulus S23 cm. Strength used in these records S21 cm. Stimuli *a* 1 and 2, *b* 1, and *c* 3, falling in late systole result in increased extent of contraction. Stimuli *c* 4 and 5 and *d* 6 show this phenomenon to be due to added contractions. Stimuli *b* 2 and 3, *c* 1 and 2, *d* 1 and 2, *d* 3, 4, and 5, *e* 4, and *e* 6 and 7 are each ineffective and show the presence of an absolute refractory period. *d*, same heart ninety-one min. after first injection; 14 c.c. injected; threshold stimulus S21 cm., stimulus used S18 cm. *e*, same as *d*, only later stage of poisoning; threshold stimulus S19 cm.; stimulus used S18 cm. *f*, tracing of Rohde's figure 2 *b*, p. 108. *g*, contractions from undrugged heart used later for *a*, *b*, *c*, *d*, and *e*.

stimuli, stimulus 14 instead of simply augmenting the already increased extent of the contraction, is followed by an extra contraction. If all of the above stimuli are effective, as above stated, such a condition would lead to the conclusion that the absolute refractory period had entirely disappeared, or that the absolute refractory period was masked by the successive contractions of separate parts of the muscle, the stimuli which were apparently effective during systole being in reality only effective for those parts in diastole. That is, the base of the ventricle, because of the slow propagation of the contraction wave, was passing into diastole at a time at which the apex was in systole. An extra stimulus for the base would therefore be effective,

and the new systole would either augment the extent of the contraction or would result in an extra summated contraction, depending upon the manner in which the new contraction wave overlapped the previous wave.

Fig. 3, *a b c*, represents a heart in about the same stage of poisoning as that represented by Fig. 3, *f*. The heart following stimuli 1 and 2 of *a*, 1 of *b*, and 3 of *c*, especially the latter, responds with contractions of increased height. Contraction 5, *c*, is analogous to the third contraction of Fig. 3, *f*, and contractions 1 and 2, *a*, and 1, *b*, illustrate the same phenomena. It will also be noticed that in Fig. 3, *a*, contractions 6, 7, and 8 show a gradual rise which may be due to stimuli 3, 4, and 5, but in Fig. 3, *b*, contractions 2, 3, and 4 following a compensatory pause show a similar increase in height, whereas in Fig. 3, *c*, contractions 1, 2, 3, and 4 do not follow a pause, yet show no difference in height, although stimuli 1 and 2 fall in the period of systole at a time corresponding to stimuli 4 and 5 respectively of Fig. 3, *a*. This would seem to indicate, therefore, that stimuli 3, 4, and 5 of Fig. 3, *a*, are ineffective, and that the increase in height is due to treppe. Furthermore, the evidence that stimuli are ineffective during the earlier part of the period of systole is to be found in such a record as 3, *d*. The heart was not stimulated during the second contraction, but in the first, third, and fourth contractions, stimuli 1 and 2, 3, 4 and 5, and 6 were applied to the heart during its period of systole, as represented by contractions 1, 3, and 4 respectively. Stimulus 6, during the time of late systole, was effective, but stimuli 1 and 2, 3, 4 and 5 at an earlier period of systole were ineffective. It would seem, therefore, from these experiments, that in Fig. 3, *f*, copied from Rohde, it is not stimuli 4, 5, and 6 of the third contraction, and stimuli 10, 11, and 12 of the fifth contraction, but stimuli 7 and 13 which cause the increase in the extent of contractions 3 and 5 respectively. Therefore the absolute refractory period, though shortened, is still present, and corresponds at this stage of the poisoning to the greater part of the systolic period.

Thus far I have laid particular stress upon the fact that the shortening of the absolute refractory period and the degree of poisoning seem to bear no definite ratio to each other and that after the first sudden shortening of the absolute refractory period it extends over a certain definite portion of the systole despite the gradual loss in contractility. Let us now consider the more advanced stages of poisoning at a time at which the force of the contraction has been greatly reduced.

Fig. 4, *a*, illustrates records of normal contractions, and *b* records from the same heart seven minutes after injecting something over 2 c.c. of the solution. It will be noticed that in the case of the simple contractions 5 and 6, the extra stimulus during systole was ineffective. In the compound contraction 7, the extra stimulus has fallen in the shortened absolute refractory period.

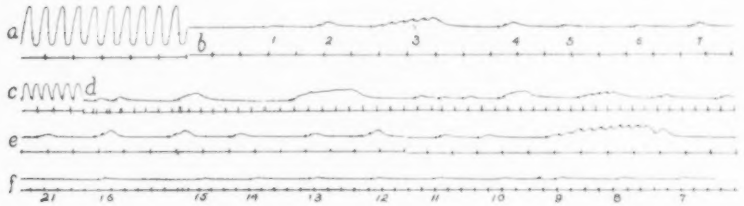

FIGURE 4. — About four-sevenths the original size. *a*, Rana cetesbiana (medium size), undrugged heart. *b*, 7 min. after intravenous injection of 2-4 c.c. of 0.75 per cent chloralhydrate; stimulus, S13 cm. *c*, R. cetesbiana, undrugged heart. *d*, same as *c*, 10–15 min. after intravenous injection of 5 c.c. of solution. *e*, Last stages of poisoning. *f*, very late stage of poisoning, illustrating All or None Law. In contractions *b*, 5 and 6, *e*, 4, 5 and 6, the second stimulus of each is ineffective. In contractions *b*, 4 and 7, *d*, 11, and *e*, 3, the second is an added contraction. Contractions *d*, 3, 4, and 8 due to fibrillary twitches called forth in each case by a single strong stimulus.

In Fig. 4, *e*, owing to the long latent period and the slowness of the contraction, it was possible to exercise a greater range of choice as to the moment of stimulation than in Fig. 4, *b*. The absolute refractory period is shortened as shown in the second and third contractions, yet there is a definite refractory period that remains even at this very late stage of poisoning. (See contractions 4, 5, and 7, Fig. 4, *e*.)

Fig. 4, *c*, *d*, illustrates another phase of poisoning. The records were taken from a heart that was very irritable, and early in the course of the experiment, before the first injection, showed a marked decrease in the force of its contractions. There was then injected from 4 to 5 c.c. of chloralhydrate solution with the result that the heart soon ceased to yield spontaneous contractions. Record *d*, contractions 3, 4, and 8 show extra contractions in response to stimuli late in the systole. By reason of the magnification used, the lever moved so rapidly that it was impossible to exercise much choice as to the moment of sending in an extra stimulus during the short systole. The extra response, furthermore, was made up of fibrillary twitches which, when viewed through a hand lens, seemed to be limited to the region

of the electrodes. These few twitches resulted in a kind of contracture, so that in this case there apparently was no absolute refractory period. However, I can confirm Rohde's statement to the effect that, "Bei starkeren graden der Vergiftung ist die refraktäre Period anscheinend ganz verschwunden," if particular emphasis be laid on his proviso, "anscheinend."

While in certain cases it may appear that the heart responds to single induction shocks in any part of the extremely short systole, yet by reason of the small amount of muscle which is brought into play in producing such a small contraction, it is just as probable that the second stimulus calls into action fibres which were but slightly affected by the first stimulus. In this case there would still be present an absolute refractory period which, however, would be masked by the contraction of a different set of fibres in response to the second stimulus, the effect of this stimulus being recorded during the systole of the first set.

In spite of the evidence in my experiments, which favors the idea that the refractory period persists as long as the contractility, let us grant that it may disappear at a time when the contraction is so faint as to make it practically impossible to prove its presence, of what significance would it be in support of the neurogenic theory? If, as Rohde maintains, chloralhydrate affects the nervous mechanism before it does the muscle, and the refractory period depends for its existence upon the intrinsic ganglia, then we ought to expect it to disappear with rhythmicity or at least long before the contractility. I have proven conclusively that the refractory period persists at least until the muscle has lost its rhythmicity, and with the exception of a slight shortening remains intact until the contractions are so faint that it is impossible to experiment successfully during the systolic phase. As I shall try to show later, it is not necessary to adopt Rohde's assumption in order to explain the effect of chloralhydrate.

The All or None Law.

In the chloralized heart, as in the normal heart, an increase in the strength of the stimulus is not accompanied by an increase in the extent of the contraction. Preparations in various stages of poisoning were tested, yet in no instance was it possible to confirm satisfactorily Rohde's statement to the effect that the all or none law is done away with. Variations in the height of the contractions were met with,

but these seemed to be due to treppe. The same may be said regarding such a phenomenon as is illustrated by Rohde's Fig. 5, p. 111, in which the extra systole resulting from an artificial stimulus during the time of late diastole was higher than the preceding spontaneous contractions. This increase in the extent of the extra contraction is commonly met with in the normal heart, and can be easily explained as the effect of treppe.

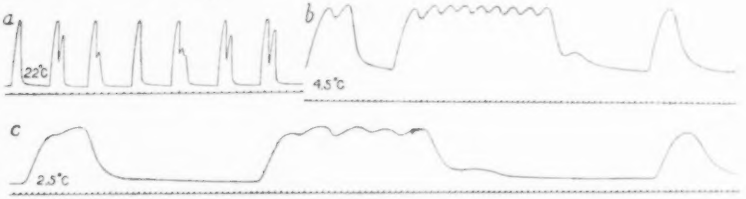

FIGURE 5. — About one-fourth the original size. *a*, *b*, and *c* show records taken from a strip of ventricular muscle (*P. elegrans*), which was first immersed in a solution of 0.7 per cent sodium chloride and 0.025 per cent calcium chloride. When the solution was drawn off, the muscle was stimulated while in a moist chamber, the air of which was cooled. (*a*) — taken at 22° C.; (*b*), at 4.5° C.; (*c*), at 2.5° C. Contractions 1 *b* and 1 *c* stimulate summation of skeletal muscle; contractions 2 *b* and 2 *c* stimulate incomplete tetanus of skeletal muscle.

In Fig. 4, *f*, a tracing is given which shows that at a very late stage of the poisoning, increase in the strength of the stimulus does not result in an increase in the extent of the contraction.

I am unable to reconcile the difference in the results as indicated by Fig. 4, *f*, and Rohde's Fig. 4, p. 110, this figure appearing the same as mine except that an increase in the strength of the stimulus is followed by an increase in the height of the contraction. In conclusion, I can only say that my data lead me to conclude that the all or none law prevails in hearts drugged with chloralhydrate.

SUMMATION.

At an early stage of chloralhydrate poisoning of the frog's heart, it is possible to secure a compound contraction from two successive stimuli, such as is represented in Fig. 3, *c* (Fig. 1, *b*, Rohde). At the proper stage of poisoning the compound contraction thus obtained may give a curve resembling in all respects one from a summated contraction of skeletal muscle. Similar results may be obtained also from excised strips of the terrapin's ventricle after immersion in a

solution of chloralhydrate. As is well known, such a phenomenon cannot be obtained from normal heart-muscle under the usual conditions of temperature. So far as the effect of chloralhydrate is concerned Rohde states that this summation may be obtained at a stage of poisoning during which the force of contraction from a given stimulus shows no decrease in extent. In my experiments, on the contrary, it was found in all cases that the action of the chloralhydrate had caused a decrease in the extent of the individual contractions before the phenomenon of summation was obtainable. A suggestive fact observed in this connection was that in some cases strips which had been immersed in a solution of chloralhydrate and which were exhibiting the phenomenon of summation displayed a difference in contractility at the two ends. A mechanical stimulus applied at one end gave a weaker contraction than when applied at the other end. So also in the case of the drugged frog's heart a similar difference might be observed between the base and the apex of the ventricle. It seems possible, therefore, that the apparent summation following upon successive stimuli may be due to added contractions of separate parts —that is, the excitation wave aroused by the first stimulus may involve only a part of the muscle, while the second stimulus following at the end of the refractory period of the first contraction may excite the formerly quiescent part and thus give an apparent summation. Such a result, as is well known, may be obtained from a strip of ventricular muscle in which a partial block has been established by compression or by section. A similar result was obtained in a series of experiments made upon strips of ventricular muscle from the terrapin which were immersed in a solution containing 0.7 per cent of sodium chloride and 0.025 per cent of calcium chloride and which were kept at a temperature between 0° and 1° C. When such a preparation in a moist chamber and at a temperature of 1° C. is stimulated, it responds with contractions which show not only a slow systole and diastole, but are also of smaller extent than at room temperatures. If now a preparation of this kind is given two successive stimuli so timed that the second stimulus falls at the beginning of the diastole of the first contraction, a summated or compound contraction results which resembles closely, as is shown by Fig. 5, contractions *b* 1 and *c* 1, the curves obtained at an advanced stage of chloral poisoning. It is most probable that lowering the temperature to this extent depresses the metabolic processes in the heart, and possibly in this case also the wave of excitation from the first stimulus fails to involve

the whole of the muscular strip. Whether or not this suggestion is correct, the parallelism between the action of the low temperature and the poisoning by chloralhydrate is significant. The evidence would indicate that the ability to give a summated contraction is connected in some way with a slowing and weakening of the normal metabolic processes within the muscle itself.

Tetanus.

If the normal heart be stimulated during the time of early diastole by single break shocks such as will call forth an extra systole, and

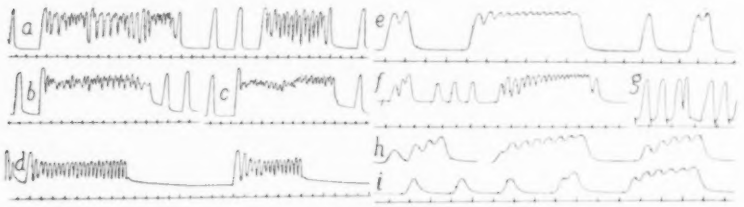

Figure 6. — About four-ninths the original size. *a*, *b*, *c*, *d*, *e* records taken from same heart. *a*, undrugged, showing effect of successive stimuli. *b* and *c* show effect of rapid tetanic stimuli. *d*, heart short time after an intravenous injection. *e*, about forty minutes after injecting something over 3 c.c. of solution. *f*, *g*, *h*, *i*, records from another heart. *f*, later stage of poisoning than that represented by *e*, *g*, normal heart showing relative diminution in extent of contraction as compared with compound contraction of drugged heart. *h* and *i*, later stage of poisoning than *f*.

this process be repeated a number of times in rapid succession, the resulting contractions are of unequal height and are usually less in extent than the first contraction of the series. The extra contractions of such a series are quick and jerky, yielding a very irregular curve, like that illustrated by Fig. 6, *a*. If tetanic stimuli of appropriate strength be employed, the ventricle may either beat at a more rapid rate or be thrown into fibrillary contractions. Should the latter happen, the form of curve then resembles that of Fig. 6, *b* and *c*.

The reaction toward tetanic stimuli in the chloralized heart is very different. The muscle is more sluggish even in the early stages of the poisoning; the tendency of the ventricle to pass into fibrillary contractions, following successive stimuli of moderate strength, gradually disappears; and instead of tetanic stimuli resulting in a series of irregular contractions, as illustrated by Fig. 6, *a*, *b*, and *c* from the normal heart, the extra contractions are of more uniform height, see

Fig. 6, *d*. As the poisoning advances, the height of the extra contractions exceeds the height of the first contraction of the series, the number of stimuli required to maintain this height being very small. In still more advanced stages of the poisoning, when contractility has been greatly impaired, the so-called tetanus curve ascends rather than descends, see Fig. 6, *e*, *f*, *h*, and *i*. Rohde's[1] Figs. 1, *b*, 6, *b*, 7 and 9 are excellent, and show in addition to the illustrations given in this paper so-called tetanus curves in which the individual contractions have been more or less fused. Strips of terrapin heart treated with Ringer's solution plus an excess of calcium chloride also show summated contractions, a slightly shortened absolute refractory period, and a marked tendency to yield compound contractions resembling summation and incomplete tetanus, these being preceded by a decrease in the extent of the contraction. Strips treated with Ringer's solution saturated with ether show similar phenomena, but so-called summation and incomplete tetanus are less marked, and it is difficult to secure curves resembling complete tetanus until after considerable diminution of contractility.

Walther[2] observed that conditions which favor the production of so-called tetanus and summation of the frog's heart likewise favor the production of treppe. I also have noticed a marked tendency to treppe in the chloralized heart at the time that it is exhibiting the phenomena of summation and tetanus. Whatever may be the underlying conditions necessary to the production of each of these phenomena, it seems evident that they are closely related. For it is possible to conceive of the so-called tetanus curves as being composed of a series of individual contractions showing a distinct treppe, which is due either to a general improvement in the irritability of the heart-muscle or to an increased conductivity whereby the entire musculature is thrown into co-ordinated action instead of responding with more or less localized contractions.

It is important to bear in mind that these compound contractions are, as a rule, less in height than the normal contractions of the undrugged heart. Provided the poisoning is not carried too far, it can be shown that the muscle may be revived so that it will, by appropriate treatment, again yield contractions that are normal and higher than the compound contractions. If the normal contractility is regained, the muscle ceases to yield contractions which simulate sum-

[1] ROHDE: *Loc. cit.*, pp. 107, 112, 113.

[2] WALTHER: Archiv für die gesammte Physiologie, 1900, lxxviii, p. 597.

mation and tetanus. It is evident, therefore, that these compound contractions depend for their existence upon a condition of the muscle which produces reduced or partial contractions in response to single induction shocks. What the exact nature of the process may be, it is at present impossible to say. As was mentioned in discussing summation, however, the results closely resemble the condition produced by low temperatures.

That which is called tetanus and summation by Rohde may be explained as a phenomenon due, in the first place, to a retardation of the process of diastole; in the second place, to an improved conduction and irritability of the muscle following upon repeated stimulation. In any event, it must be recognized that this so-called tetanus of heart-muscle differs from that of skeletal muscle inasmuch as the chloralized heart continues to exhibit a definite refractory period extending over the greater part of the phase of the contraction.

Summary.

1. The ventricular muscle of the frog or terrapin when dosed with chloralhydrate shows a gradual diminution in contractility. During the earlier stages of the poisoning the irritability increases, after which it gradually diminishes. The spontaneous beats become weaker and finally disappear. Eventually the muscle loses all irritability to the strongest induction shocks.

2. During the progressive stages of the poisoning the refractory period is at first somewhat shortened, so that the muscle becomes irritable to artificial stimulation toward the end of systole. There remains, however, a definite refractory period, designated in this paper as the shortened absolute refractory period, which extends over most of the systolic phase and is retained as long as the heart shows any response to artificial stimulation.

3. At a certain stage in the poisoning the heart-muscle when stimulated by two successive shocks exhibits the phenomenon of summated contractions and gives on repeated stimulation a curve resembling that of incomplete tetanus of skeletal muscle. In the later stages of poisoning this tetanic curve may show a gradual rise.

4. The maximum shortening reached by these so-called compound contractions is always less than that shown by the normal single contractions of the unpoisoned muscle.

5. Similar phenomena of summated contractions and compound contractions resembling tetanus may be obtained from heart-muscle

that is cooled to near zero centigrade, when treated with ether, or when treated with an excess of calcium. In all such cases the individual contractions are first diminished in extent before the phenomenon of summation is exhibited. The view is suggested that this summation may be due to an improved condition of conductivity brought on by successive stimuli whereby the entire musculature is brought into action.

6. The characteristic properties of the heart-muscle are not lost as a result of the action of chloralhydrate. A definite refractory period remains, and the all or none law holds true at the time when the heart shows the characteristic effects of the poisoning, such as complete loss of spontaneous beats and the appearance of the phenomenon of summated contractions.

RECORD IN DETAIL OF THREE EXPERIMENTS.

Experiment 77, April 3, 1906. — Rana cetesbiana (very large ♀).

3.00–3.33 P.M. Pithed and fastened to cork sheet as described under head, "Method."

3.36. Rate of rhythm equals $28\frac{5}{8}$ beats per minute; height of contraction equals 8.7 mm.

4.3. Secondary coil 20 cm. from primary; no response to first nine stimuli; to tenth stimulus, response late in diastole; later response one-fourth length of diastole from tip of crescent.

4.18. Rate, $22\frac{1}{8}$ per minute; height, 10.7 mm.

4.21½. Injected into anterior abdominal vein 3 c.c. of 0.75 per cent solution chloralhydrate (Eimer and Amend). Height of contraction is immediately decreased; systole lengthened; rate of rhythm slowed.

4.23–4.27. Distinct summation; S22 cm.[1] Absolute refractory period shortened (?); height, 8–8.8 mm.; rate, 10.9 per minute.

4.28½. Rate, 13.6 per minute; height, 8 mm.; responds to S24 cm.

4.28½+. Ceases to respond to S24 cm., responds to S23 cm.

4.37. Rate, $19\frac{7}{15}$ per minute; S23 cm. no response, S22 cm. response late in D; S21 response one-third in D.

4.40. Height of contraction from artificial stimulus higher than that of spontaneous contraction.

4.45. Distinct treppe in response to successive artificial stimuli.

4.51. Responds to S23 cm., one-third in D.

4.54. Responds to S24 cm., one-third in D, distinct summation. Rate, $20\frac{3}{8}$ per minute; height, 7.2 mm.

5.00. Height, 7.6 mm.; responds to S24 cm., one-fifth in D.

[1] S22 cm. equals secondary coil 22 cm. from primary.

5.4. Rate, $21\frac{3}{5}$ per minute; height, 7.9 mm.; responds to S25 cm., one-fourth in D.

5.6. Rate, $21\frac{6}{11}$ per minute; height, 8.1 mm.; responds to S25 cm., one-fourth in D.

5.8. Rate, 22.5 per minute; height, 8.1 mm.

5.11. Rate, $21\frac{1}{2}$ per minute; height, 8.7 mm.

5.17. Rate, $21\frac{1}{2}$ per minute; height, 11.5 mm.

5.40. Responds to S25 cm., very near beginning of D.

5.48. Responds to S29.5 cm., very near beginning of D.

5.49. Responds to S30 cm., but uncertain, not always calling forth an extra contraction.

5.53. Responds to S30 cm., but less certain than at 5.49.

5.57. Rate, 23.5 per minute; height, 11.9 mm.

5.58. Injected 1 c.c.

5.59½. Rate, 22.2 per minute; height, 10.5 mm.; responds to S30 cm.

6.5½. Rate, 21.7 per minute; height, 12 mm.; responds to S29 cm., one-fourth in D.

6.15½. Rate, $22\frac{1}{11}$ per minute; height, 11.7 mm.; ceased to respond to S29 cm.

6.18. Responds to S28 cm. in beginning of D.

6.21. Rate, 22.5 per minute; height, 11.7 mm.

6.22. Injected 1 c.c.

6.25. Rate, $20\frac{1}{2}$ per minute; height, 9.8 mm.; responds to S28 cm. toward end of D; summation marked; latent period short.

6.28. Rate, $19\frac{2}{3}$ per minute; height, 10.7 mm.; responds to S29 cm. in beginning of D.

6.43. Rate, $20\frac{6}{7}$ per minute; height, 10.7 mm.; ceases to respond to S29 cm., but responds to S28 cm., one-fourth in D.

6.48. Injected 2 c.c.

6.50. Rate, 20 per minute; height, 10.2 mm.; responds to S28 cm. in beginning of D; form of curve is changed, slow systole, rapid diastole.

6.55. Rate, $17\frac{2}{3}$ per minute; height, 11.7 mm.; responds to S28 cm., but not to S28.5 cm.

7.2. Ceases to respond to S27.5 cm.

7.13. Rate, 18 per minute; height, 11.9 mm.; responds to S27 cm., one-third in D

7.15. Injected 2 c.c.

7.17. Rate, $15\frac{3}{4}$ per minute; height, 11.8 mm.

7.20. Rate, 15 per minute; height, 12.2 mm; responds to S27 cm., but not to S28 cm.; compensatory pause lengthened.

7.31. Injected 1 c.c.

7.46. Responds to S24 cm., but not to S25 cm.

7.52. Preparation, in response to successive stimuli, yields a compound contraction resembling incomplete tetanus.

8.00. Injected 2 c.c.

8.1. In response to number of successive stimuli heart assumes a rhythm of 32½ per minute; height, 8.9; changes to slower rhythm.

8.40. Rate, 3 per minute; height, 13.5 mm.; cardiogram more or less flat-topped.

8.44. Rate, 20 per minute.

8.44–9.43. Heart ceases to beat spontaneously; does not respond to S22 cm.; summation; excellent compound curve resembling tetanus.

10.30. Coil 22, 20, 18, 16, 14, 12, 10, 8, 6, 4, 2, 0, —15, all contractions same height; refractory period does not infringe upon the systole further than at 4.27.

April 26, 1906. — Rana cetesbiana.

11.00 A.M. Frog pithed as described in "Method."

11.3. Rate, 32 per minute; height, 10.9 mm.

11.22. Rate, 27.5 per minute; height, 10.1 mm.

11.23. Injected 2 c.c.

11.23. Rate, 26.2; height, 7.6 to 5.4 mm.

11.24. Rate, 18; height, 4.9 to 6.5 mm.

11.24½. Heart stops; does not respond to S19 cm.; but does to S18 cm.

11.25. In response to S17 cm. marked summation; absolute refractory period not shortened.

11.27½. Response to S18 cm.; begins to beat spontaneously. Rate, 17.5 average height, 8.8.

11.29. Rate, 20.6; height, 9.1 mm. In response to S17 cm. A. R. P. slightly shortened.

11.38. Responds to S20 cm.

11.42. Rate, 25.5; height, 10.3; responds to S21 cm.

11.45½. Rate, 26.2; height, 10 mm.

11.46. Injected 2 c.c.

11.48½. Rate, 27.2; height, 10.1 mm.

11.50. Injected 2 c.c.

11.53. Injected 4 c.c.; rate, 9.5; height drops from 9.8 to 4.7, and later to 3.5 mm.

11.55½. Rate, 15; height, 5 mm.; marked summation in response to S21 cm.

11.57. Muscle readily goes into so-called tetanus.

12.00 M. Responds to S23 cm., but not to S24 cm.

12.29 P.M. Rate, 20; height, 9 mm.

12.42. Injected 2 c.c.

12.42+. Rate, 3 per minute; height gradually decreased thus 9, 8.3, 4.2, 1.9, 0 mm.

12.44. Responds to S22 cm. Height, 1.4 mm.

12.45. Ceases to respond to S22 cm.

12.46½. Spontaneous contraction after long intervals of rest.

12.48. Height, 5.1 mm.; summation marked; A. R. P. normal.

12.53. Rate, 6 per minute; height, 3 mm.

1.3. Responds to S24 cm., but not to S25 cm.

1.5. Responds to S21 cm., but not to S22 cm.

1.7. Injected 1 c.c. Rate is slowed; height decreased; A. R. P. shortened as at 11.29; latent period lengthened.

1.30. Definite refractory period as at 1.07 in response to S16 cm.

1.32. Injected 1 c.c.; ceases to beat spontaneously; in response to S16 cm. contraction very weak.

2.15. Responds to S18 cm. but not to S19; definite refractory period; height, 0.5 mm.

Experiment 81, April, 1906. — Rana cetesbiana (medium sized).

10.00 A.M. Frog pithed and fastened in place as described in method.

10.16. Rate, 50.4 per minute; height, 10.8 mm.; responds to S25 cm. one-third in D.

10.18. Rate, 52.5 per minute; height, 10.2 mm.; responds to S25 (uncertain).

10.24. Injected 2 to 4 c.c. In course of 20 seconds, height is decreased from 9.8 to 5 mm., then ceases to beat for 38 seconds, after which a single contraction is followed by a pause of 18 seconds. The heart now continues to beat for a time at a rate of 30 per minute; height, 3.3 mm.; slight increase in tone.

10.27. Rate slower, contractions very feeble.

10.31. Height, scarcely 0.5 mm.; refractory period still present even with S13 cm.; ceases to beat spontaneously.

10.38. Strong stimuli call forth weak contractions; refractory period still present.